



Etude des effets respectifs de l'âge et de l'hypertension sur l'anatomie et la fonction des artères centrales et périphériques

David Rosenbaum

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Equipe 2 : Imagerie du flux, de la fonction cardiaque, de la microcirculation et des échanges
tissulaires

**Etude des effets respectifs de l'âge et de l'hypertension sur
l'anatomie et la fonction des artères centrales et
périphériques**

Par David Rosenbaum

Thèse d'Université pour l'obtention du titre de docteur de l'Université Paris 6

Dirigée par Pr. Xavier Girerd et co-encadré par le Dr. Alban Redheuil

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Devant un jury composé de :

Monsieur le Pr Stéphane Hatem	Président
Monsieur le Pr Pierre Lantelme	Rapporteur
Monsieur le Pr Bernard Levy	Rapporteur
Monsieur le Pr Jean Michel Halimi	Examineur
Monsieur le Dr Alban Redheuil	Co encadrant
Monsieur le Pr Xavier Girerd	Directeur de thèse

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« Tous les phénomènes observables peuvent être (...) disposés de telle manière que l'étude de chaque catégorie soit fondée sur la connaissance des lois principales de la catégorie précédente, et devienne le fondement de l'étude de la suivante. Cet ordre est déterminé par le degré de simplicité ou, ce qui revient au même, de généralité des phénomènes, d'où résulte leur dépendance successive et par suite la facilité plus ou moins grande de leur étude. »

— A. Comte, Cours de philosophie positive, deuxième leçon

Introduction

Les progrès récents de la médecine, le traitement des maladies infectieuses, les progrès de la cardiologie interventionnelle et du traitement des cancers en particulier ont fait que vieillir est devenu un phénomène de plus en plus fréquent dans la population française où depuis les années 50 il est possible de vivre encore plus longtemps chaque année. Mais le vieillissement est un dénominateur commun à beaucoup de maladies chroniques telles que le diabète, l'hypertension ou le cancer. C'est aussi un facteur de risque cardiovasculaire à part entière très important et il est donc communément et médicalement admis qu'il est très risqué de vieillir. Cependant nous ne sommes pas égaux devant le vieillissement et il existe une hétérogénéité importante chez les personnes âgées au niveau de leurs capacités intellectuelles, de leur autonomie et donc aussi de leur état vasculaire.

En parallèle, la découverte des facteurs de risque cardiovasculaire et de l'hypertension en particulier a permis de mettre à jour une intrication complexe de mécanismes entre le vieillissement et les conséquences de ces facteurs de risque sur le système cardiovasculaire. Au cours des ans, les progrès technologiques ont permis à de nouvelles techniques de pénétrer de plus en plus profond dans l'analyse des processus moléculaires et physiologiques sous-jacents aux modifications anatomiques et fonctionnelles observées d'abord de façon macroscopique, puis microscopique et moléculaire. En particulier, une des grandes évolutions a été le passage à une technologie permettant des investigations non invasives et donc ainsi à une étude de la physiologie in vivo.

C'est dans cette lignée de travaux que s'inscrit notre travail de thèse qui a cherché à étudier les conséquences de l'âge et de l'hypertension en utilisant des nouvelles techniques d'imagerie non invasive avec une attention particulière au territoire microvasculaire.

1 Rationnel

1.1 Hypertension et système artériel

1.1.1 Hypertension et microcirculation

Les artères de résistance jouent un rôle crucial dans le contrôle de la pression artérielle (PA), en effet, c'est à leur niveau que se produit la principale baisse de la pression. Les résistances périphériques dans les petites artères (diamètre luminal <350 µm) et artérioles (diamètre luminal <100 µm) représentent 45% à 50% de la résistance périphérique totale, tandis que les capillaires (≈7 µm de diamètre) comptent pour 23% à 30% [1,2]. Selon la loi de Poiseuille de légères altérations de lumière artérielle, soit fonctionnelles ou structurales, entraînent des changements importants de résistance et donc de pression.

1.1.1.1 Aspects structurels

L'atteinte des artérioles dans l'hypertension essentielle est caractérisée par les phénomènes suivants : vasoconstriction, remodelage eutrophique avec augmentation du rapport media/lumière (MLR) et raréfaction capillaire. La majeure partie des modifications structurelles observés chez les patients présentant une hypertension essentielle consiste en un remodelage eutrophique [3]. Ce remodelage eutrophique centripète correspond à un épaississement de la média, une réduction de la lumière et du diamètre extérieur du vaisseau sans modification de la quantité totale de tissu de la paroi. Il s'ensuit donc une augmentation du MLR et l'impression d'un remodelage « vers l'intérieur » d'où son nom de remodelage eutrophique centripète [4]. Selon l'équation de Lamé (stress de la paroi périphérique, $\sigma_\theta = \text{MBP} \times R / h$, où MBP est la pression moyenne R est le rayon, et h est l'épaisseur de paroi), un réarrangement de la même quantité de matériau du mur autour d'une lumière réduite sans croissance nette des cellules permet de normaliser le stress pariétal, un des stimuli de l'hypertrophie [5]. Ce remodelage a été décrit comme un des premiers stades du retentissement artériel de l'hypertension essentielle [6]. Il est dit spécifique de l'hypertension essentielle parce que les artères de résistance chez les patients souffrant d'hypertension secondaire [7] sont-elles caractérisées par un remodelage hypertrophique avec épaississement de la média, diminution de la lumière et surtout augmentation de la quantité totale de tissu de la paroi médié par une hypertrophie et une hypertrophie des CML [8]. Avec l'hypertension il

a été spéculé mais non observé qu'un remodelage eutrophique puisse évoluer vers un phénotype hypertrophique. Chez les patients présentant une hypertension essentielle, le remodelage eutrophique pourrait représenter un mécanisme de protection contre l'élévation de pression, empêchant l'augmentation du stress pariétal au niveau d'artérioles ou de capillaires qui seraient incapables de résister.

Chez l'homme, la méthode de détermination du MLR de référence consiste à avoir recours à des artérioles disséquées de biopsie glutéales puis montées sur un fil de myographe. Une alternative non invasive a été récemment apportée par la mesure du rapport mur/lumière (Wall-to-Lumen-Ratio, WLR) des artérioles rétinienne en utilisant une technologie de balayage laser Doppler. Ce WLR a été montré comme significativement augmenté chez les patients hypertendus jamais traités [9]. Il a de plus été significativement corrélé avec le MLR des artérioles sous cutanées [10].

La raréfaction correspond à la réduction du nombre de petites artères interconnectées et des capillaires [11]. La mesure de la raréfaction capillaire raréfaction dans l'hypertension essentielle humaine a été obtenue par capillaroscopie in vivo de la microvasculature périunguéeale. Il existe une raréfaction fonctionnelle (augmentation du nombre de microvaisseaux non perfusés) qui peut progresser vers raréfaction structurelle (absence anatomique de microvaisseaux). Au niveau rétinien, il n'existe pas de données quantifiées sur la raréfaction des capillaires par méthodes non invasives.

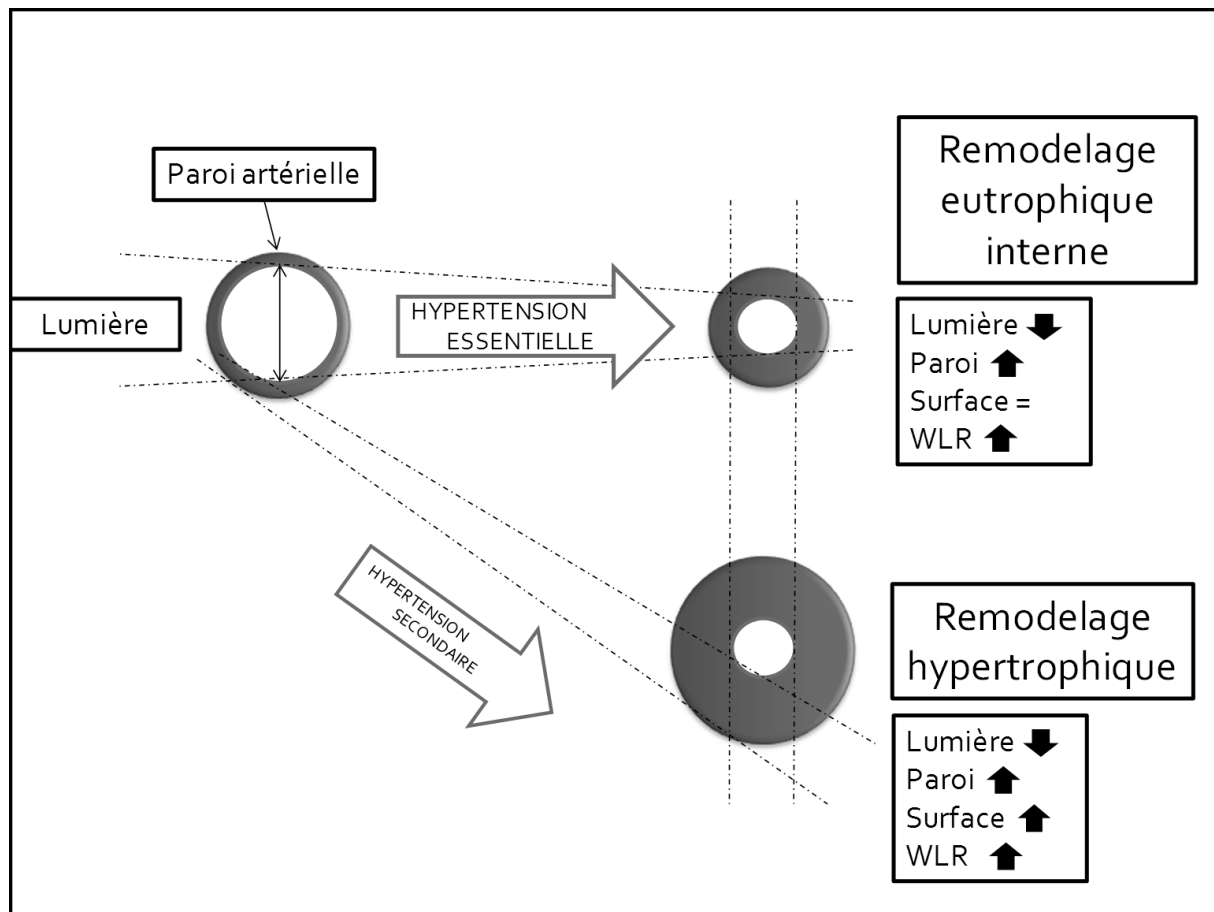


Figure 1 Aspects du remodelage microvasculaire dans l'hypertension artérielle

1.1.2 Hypertension et macrocirculation

1.1.2.1 Remodelage artériel

Dans l'hypertension essentielle, le remodelage des grosses artères est caractérisé par une augmentation de l'épaisseur intima-média (IMT) et par un élargissement de la lumière des artères élastiques proximales sans changement dans le diamètre de la lumière des artères musculaires distales [12]. Cette augmentation [13] permet de compenser l'augmentation de la pression et tend à normaliser le stress pariétal selon l'équation de Lamé ($\sigma_{\theta} = P \times R/h$, où le stress est proportionnel au rayon (R), à la pression (P), et inversement proportionnelle à l'épaisseur (h)).

L'élargissement des artères élastiques proximales a été largement décrit chez l'homme dans les études utilisant principalement des ultrasons [14,15]. Il est généralement attribué à la rupture des fibres d'élastine secondaire à l'effet de fatigue par les contraintes constantes et pulsatiles imposées par les battements cardiaques [16,17] et la pression constante. Toutefois,

des mécanismes de croissance et d'apoptose des CML pourraient également être impliqués. Le diamètre de la racine aortique a été étudié chez des hypertendus et il a été constaté que plus la pression pulsée augmentait, plus le diamètre était élevé, renforçant ainsi l'hypothèse d'une causalité de la pulsatilité. Il a été possible de montrer en IRM un lien entre la pression pulsée centrale et l'augmentation de diamètre de l'aorte ascendante, de la longueur et de la largeur de la crosse [18]. S'il existe des preuves sur le rôle de la pression pulsée sur l'élargissement de la racine aortique, les conséquences de ce remodelage sur la pression centrale restent peu claires [14,19]. Ainsi, à l'inverse de ce qui a été constaté chez les hypertendus, une relation négative entre la pression pulsée et le diamètre de la racine aortique a été observé chez des normotendus [14]. Au total, ces données suggèrent qu'une pulsatilité excessive favoriserait l'élargissement de l'aorte lorsque la paroi artérielle est déjà endommagée, par exemple par la maladie hypertensive [14]. En revanche, lorsque la paroi aortique est saine, un volume réduit de l'aorte pourrait augmenter l'impédance caractéristique et générer des ondes de réflexion, et donc élever les pressions pulsées et systoliques centrales. Ainsi, chez les patients hypertendus, épaissement de la paroi, augmentation de pression et élargissement de la lumière se conjuguent pour moduler le stress pariétal.

1.1.2.2 Rigidité des grosses artères

1.1.2.2.1 Définition et rappel

Le terme de rigidité est un terme générique pour désigner la perte de compliance et les modifications des propriétés de la paroi artérielle. La compliance des grosses artères et en particulier celle de l'aorte, représente leur capacité à altérer la pulsatilité de l'éjection ventriculaire pour la transformer en un flux et une pression continus en aval afin de permettre l'irrigation des organes à basse énergie. Durant la systole ventriculaire, une partie du volume éjecté est directement transmise, l'autre stockée momentanément dans l'aorte et les grosses artères élastiques étirant les parois et générant une augmentation de pression locale. Ainsi une partie de l'énergie de l'éjection est divertie vers la paroi artérielle. Durant la diastole, cette énergie permet le « recoil » de l'aorte (c'est-à-dire sa recontraction à son niveau basal) pour éjecter le volume accumulé et ainsi permettre un flux continu vers les tissus périphériques. L'efficacité de ce mécanisme dépend de la rigidité et de la géométrie de ces artères [20][21]. En cas de rigidité basse, la pression est basse mais dans l'hypertension ce système est rigide et nécessite une plus forte énergie pour se distendre et être efficace. Ainsi une plus grande partie

du volume d'éjection va aller directement vers l'aval avec 2 conséquences : diminution du flux diastolique et augmentation de la pulsatilité.

1.1.2.2.2 Physiopathologie

La rigidité aortique est un trait dont le caractère hérité est modéré et dont les mécanismes génétiques sont encore incomplètement compris [22].

La média et ses composants jouent un rôle capital dans la détermination des propriétés élastiques de la paroi artérielle. L'unité structurelle de base de la média des artères élastiques est l'unité lamellaire (ou complexe musculo élastique) décrit par Wolinsky et Glagov en 1967 [23]. Elle est constituée d'une couche centrale de CML séparée de chaque côté par des fibres d'élastine et par une couche de matrice extracellulaire. Cette matrice est composée de protéoglycanes et de collagène. Les éléments fibrillaires de la matrice sont hautement connectés aux CML via des plaques denses et des points d'adhésion focaux permettant ainsi la transduction du signal d'étirement aux CML. Ainsi, sans changer fondamentalement la structure de la paroi, une modulation de contractilité des CML peut moduler sa rigidité. Les fibres d'élastine sont étroitement intriquées avec des fibres de collagène I, III et V qui sont enroulées.

Un grand nombre de mécanismes peuvent influencer la rigidité de la paroi artérielle. Par exemple, l'activation du système rénine angiotensine, l'inflammation ou les calcifications, l'implication du système immunitaire, ou la rigidité des CML elles-mêmes. Une autre hypothèse est l'amplification des phénomènes de fatigue par l'augmentation de la pression artérielle avec une destruction/désorganisation progressive des fibres d'élastine et le renforcement en collagènes rigides [24]. Plusieurs études sont venues infirmer cette hypothèse en montrant une rigidité moindre accompagnant une paroi épaisse chez des patients hypertendus [25] ou chez des rats [26] spontanément hypertendu ce qui plaide en faveur d'un mécanisme adaptatif à l'élévation de pression.

1.1.2.2.3 Effets de la pression

Lors d'une augmentation de pression, même rapide, les fibres de collagènes rigides sont progressivement recrutées et déroulées ce qui augmente la rigidité du vaisseau. Ainsi la distensibilité est moindre en cas de pression augmentée mais pour une même augmentation de

pression, la distensibilité diminuera beaucoup plus pour des pressions basses au départ. Chez des patients hypertendus, la relation distensibilité/pression est modifiée avec une distensibilité plus grande de l'artère radiale pour un même niveau de pression [27] et chez des jeunes hypertendus, une augmentation de la pente de la relation module élastique/ stress témoignant d'une paroi plus rigide [24]. Ainsi cette rigidification peut être « aigue », « pression dépendante » et réversible après normalisation de la pression comme démontré sur l'artère radiale [28].

De nombreuses études cliniques portant sur des patients hypertendus ont très clairement montré l'augmentation de la rigidité aortique la pression en utilisant notamment la mesure de la VOP. Cependant l'aorte est une artère dont la structure change tout au long de son parcours et dont l'analyse segmentaire est difficile. C'est là que de nouveaux outils tels que l'IRM ont pu montrer l'importance de la distensibilité de l'aorte ascendante par rapport à la descendante [26]. Par ailleurs, les grosses artères élastiques proximales contiennent beaucoup plus d'élastine que les artères musculaires de moyen calibre qui contiennent plus de CML. Ainsi pour une VOP de $4/5 \text{ m.s}^{-1}$ dans l'aorte ascendante, elle est à $5/6 \text{ m.s}^{-1}$ dans l'aorte abdominale et $8/9 \text{ m.s}^{-1}$ dans les artères iliaques et fémorales [29]. Cette différence de rigidité génère des ondes de réflexions qui vont limiter la transmission d'énergie aux artérioles mais augmenter la pression centrale et participer à la rigidité artérielle. Ainsi dans l'hypertension on observe une augmentation importante de la rigidité de l'aorte ascendante donc une diminution du gradient de rigidité et une diminution des ondes de réflexions pouvant impacter la microcirculation.

1.1.3 Relations entre micro et macrocirculation

Il n'existe que peu de données concernant les relations entre les petites et les grosses artères dans l'hypertension. Un des concepts développé par Laurent et al [30] est celle d'une relation croisée entre ces 2 territoires avec une influence de la pulsatilité des grosses artères sur le remodelage microvasculaire. Il part de la constatation de liens entre la pression pulsée brachiale et lésions de la microcirculation des organes cibles de l'hypertension (microalbuminurie, lésions de la substance blanche) mais aussi entre rigidité artérielle et albuminurie, rétrécissement artériolaire rétinien ou déclin cognitif. La séquence proposée est résumée dans la figure 4. L'augmentation du remodelage microcirculatoire secondaire à

l'élévation de pression est renforcée par des liens observés entre pression pulsée et MLR [31] ou entre pression systolique et WLR rétinien [32]

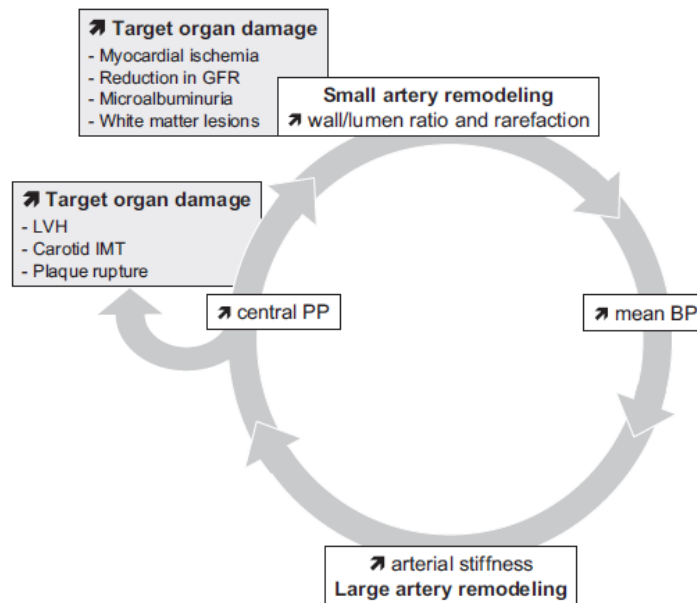


Figure 2 Représentation schématique des relations entre les altérations des territoires micro et macrovasculaires. D'après Laurent et al [30]

1.2 Vieillessement artériel

1.2.1 Vieillessement microvasculaire, aspects structurels

Le vieillissement microvasculaire implique des modifications structurelles au niveau de l'endothélium, des cellules musculaires lisses et de la matrice extracellulaire des vaisseaux [33]. Par ailleurs, l'augmentation du tonus microvasculaire liée à l'âge et l'augmentation de la pression pariétale conduit à un remodelage progressif [34]

L'artériolosclérose, due à la rigidification, avec perte d'élasticité des artérioles doit être distinguée de l'artériosclérose ainsi que de l'athérosclérose. L'artériolosclérose est caractérisée par un épaississement de l'intima, par une prolifération de cellules musculaires lisses (CML) et par un dépôt de matrice extracellulaire, entraînant une augmentation du rapport média sur lumière (media/lumen ratio, MLR). Plus tardivement il s'agira d'un remplacement des cellules musculaires lisses vasculaires par des zones de fibrose ou acellulaires [35].

Les cellules endothéliales, subissent apoptose et dégénérescence médiées par le stress oxydatif [36] avec des modifications telles que paucicellularité, affinement et allongement des cellules et épaissement de la membrane basale. Les CML sénescents, subissent une altération phénotypique, prolifèrent, migrent et remodelent la matrice extracellulaire. Elles perdent leurs propriétés spécialisées ou différenciées et deviennent prolifératives et très mobiles. Avec l'âge survient aussi une réorganisation de la matrice extracellulaire avec augmentation du collagène et fragmentation de l'élastine. Ces changements dans la teneur relative en collagène/'élastine provoquent une augmentation de la fibrose et contribuent à la rigidification de la paroi vasculaire [37]. Par ailleurs, une rigidification des CML elles-mêmes intervient via les intégrine β_1 , la fibronectine et l'actine musculaire lisse α [38]. Les péricytes jouent un rôle capital dans la microcirculation en raison de leurs rapports étroits avec les cellules endothéliales et les modifications qu'ils subissent avec le vieillissement contribuent au développement d'anomalies morphologiques et physiologiques. Il a été également démontré que dans la rétine de rats âgés que le vieillissement induisait un élargissement des capillaires et des veinules terminaux, un épaissement des membranes basales et un switch phénotypique des péricytes vers un phénotype de type CML [39] et production de collagène.

1.2.2 Vieillissement macrovasculaire

1.2.2.1 Aspect structurels

En dehors de l'athérosclérose, une observation commune et fréquemment rapportée de l'effet structurel de l'âge sur les artères est l'augmentation de l'épaisseur pariétale. Elle a été largement étudiée au niveau carotidien où l'augmentation de l'épaisseur intima média (IMT) se produit linéairement ($\sim 5 \mu\text{m} / \text{an}$) avec l'âge [40]. Cet épaissement a également été observé dans les vaisseaux périphériques [41,42]. L'ampleur de l'épaissement semble liée à l'âge mais les données sont contradictoires quant à savoir si cet épaissement est comparable entre les artères centrales et périphériques [41][43].

Une autre modification est l'augmentation du diamètre luminal. Elle a été observée au niveau des artères centrales [41] et des artères périphériques [44] avec des amplitudes relatives comparables. L'analyse de segments de section d'artères carotides communes a pu montrer une augmentation de $.017\text{mm}/\text{an}$ sur des artères saines et de $0.03\text{mm}/\text{an}$ sur des artères athérosclérotiques [45]. Au niveau des membres inférieurs, il a pu être observé une augmentation sur 40 ans du diamètre des artères poplitées et fémorales de 22 à 26% et de 12 à

21% respectivement [46,47]. Plusieurs explications ont été avancées notamment un mécanisme adaptatif à l'épaississement pour maintenir la tension pariétale, la perte/fissuration des fibres d'élastine ou l'adaptation à une rigidité du vaisseau plus importante.

Enfin, grâce à l'utilisation de l'IRM et à la validation d'outils de mesure reproductibles un allongement de l'aorte ascendante a pu être mis en évidence [18] conduisant à une arche aortique élargie de courbure moindre. La longueur de la crosse aortique augmentait en moyenne de 30% (100,4 à 130.9mm) de la 2e à la 7e décennie alors que l'aorte descendante elle n'augmentait pas de longueur.

1.2.2.2 Aspect fonctionnels

La principale modification fonctionnelle qui marque le vieillissement des grosses artères est leur rigidification.

L'aorte thoracique et les artères élastiques proximales se dilatent d'environ 10% à chaque battement contre 2 à 3% pour les artères musculaires [48]. Une telle différence permet d'expliquer les différences de rigidification qui peuvent être observées entre les artères centrales et périphériques si on considère que l'hypothèse de fatigue du matériau élastique s'applique aux tissus biologiques. En effet, en extrapolant les données disponibles pour le caoutchouc naturel, pour une extension de 10%, la fracture est calculée pour se produire à 8×10^8 cycles (correspondant à 30 ans à raison de 70 battements/min). Pour les artères périphériques avec extension de 3%, et le même nombre de cycles, la rupture ne devrait pas se produire jusqu'à plus de 3×10^9 cycles, ce qui correspond à plus de 100 années. Ainsi la fracture de lamelles élastiques peut être observée dans des vieilles aortes [49] en histologie alors que peu de changements sont observés dans des artères musculaires distales [48]. Ces ruptures d'élastine et peuvent être source à la fois d'une dilatation (après fracture du matériau de support de charge) et de rigidification (par transfert de contraintes aux composants plus rigides de collagène de la paroi artérielle).

Le vieillissement ne retentit pas sur la fonction de conduit de l'arbre artériel. Il a par contre un effet marqué sur la fonction d'amortissement. L'augmentation de la rigidité artérielle avec l'âge peut être mesurée par l'augmentation de la Vitesse de l'onde de pouls (VOP). La VOP "Aortique" est estimée de façon non invasive à partir du retard du pied de l'onde de pression en fémoral par rapport à la carotide, divisé par la distance parcourue par

l'impulsion. Une valeur typique à 20ans est de 5 m/s et 12 m/s à 80 ans [50,51]. Des valeurs semblables ont été déterminées pour l'impédance dans l'arche et l'aorte proximale [52]. L'augmentation d'impédance observée avec l'âge (x4 entre 20 et 80 ans) est en fait la double conséquence de la précocité des ondes de réflexion et de l'augmentation de la rigidité intrinsèque. Cette augmentation se voit dans l'augmentation de la pression pulsée centrale qui est plus importante que celle de la pression pulsée brachiale car ce dernier est déjà très « augmentée » dès le jeune âge. Ainsi entre 20 et 80 ans, au niveau brachial entre l'augmentation de la systolique et la diminution de la diastolique, la pression pulsée passe de 35 à 60mmHg en moyenne contre de 22 à 65mmHg pour la pression centrale [53,54].

Depuis le premier papier de Bramwell et Hill en 1922 [55] l'augmentation de la rigidité avec l'âge a été confirmée dans un certain nombre de différentes populations et en utilisant une variété de techniques et des indices de rigidité différents, incluant l'analyse des spectres d'impédance, [30] de la compliance/distensibilité aortique en utilisant l'échographie, [32] l'IRM [19] la VOP [36]. Si initialement la plupart de ces études transversales suggéraient une relation linéaire entre rigidification aortique et âge, des études longitudinales suggèrent un effet non linéaire, avec une accélération de la rigidification aortique liée à l'âge après la 50 ans [63]. En ce qui concerne les artères périphériques, l'augmentation de VOP des artères brachiale, radiale fémorale et carotides constatée est moindre et plus graduelle, peut être en raison d'un stress mécanique moindre et d'un moindre contenu en fibres élastiques.

1.3 Méthodes d'étude

1.3.1 Microcirculation

1.3.1.1 Biopsies des artérioles sous cutanées

La méthode de référence pour l'étude des propriétés structurelles et fonctionnelles des petites artères est la biopsie de tissu sous cutané glutéal d'où sont disséquées des artérioles qui sont ensuite montées sur un myographe. Le premier myographe fut développé par Bevan et Osher [64] en 1972 consistait à stimuler une artériole de 200µm montée sur 2 câbles fins clampés à chaque extrémité ce qui garantissait que la réponse observée était isométrique. Depuis, plusieurs types de myographes ont été inventés permettant l'analyse d'artérioles plus

petites, l'analyse de réponse force/vitesse ou le contrôle de la pression intra-artériolaire par sa canulation. Les diamètres analysés varient entre les myographes étant donné les différentes techniques utilisées. Ce sont les travaux de Mulvany qui ont proposé l'analyse du diamètre du vaisseau qui donne la réponse isométrique maximale. C'est avec cette méthode qu'ont été faites les observations sur le remodelage des artérioles dans l'hypertension [3] complété par la suite par la microscopie électronique [65]. Par ailleurs, étant donné son caractère invasif et non applicable à grande échelle, il n'existe pas de données prospectives montrant que les altérations visualisées avec le myographe soient reliées à un sur-risque cardiovasculaire.

1.3.1.2 Analyse des artérioles rétiniennes

Un territoire microvasculaire facilement accessible in vivo est celui de la rétine. Le classique fond d'œil manque de précision et en dehors des stades de rétinopathie hypertensive, il ne permet pas d'analyser précisément le remodelage artériel. En utilisant une caméra de haute résolution couplée à un logiciel dédié (le retinal vessel analyzer), il est toutefois possible de calculer un ratio de diamètres artères/veines [66]. Ce rétrécissement relatif des artères par rapport aux veines mesuré par une diminution du arterio/venous ratio (AVR) a pu être montré dans l'hypertension [67] mais il n'a pas été validé contre le myographe.

Une technique d'imagerie des artérioles rétiniennes de précision a été développée par l'équipe de Michelson: la scanning Laser Doppler Flowmetry. [68] Cette technique repose sur la combinaison d'une image optique (qui permet d'obtenir le diamètre total du vaisseau) et d'une image doppler (qui permet d'avoir le diamètre de la lumière circulante) [69]. Cette technique a pour résolution une quinzaine de microns et permet ainsi de mesurer non pas le media/lumen ratio mais le Wall/lumen ratio qui prend en compte tout la paroi artériolaire. De nombreux travaux ont permis de la valider en montrant notamment une corrélation entre les remodelages observés dans la rétine et avec le myographe [70]. Par ailleurs, une élévation du WLR a été montrée chez les hypertendus [71], chez les patients avec ATCD cérébrovasculaires [72] ou avec microalbuminurie [73]. Plus récemment, elle a permis d'observer un remodelage hypertrophique chez des patients présentant un hyperaldostérionisme [74].

Cette approche rétinienne n'a pas encore permis de montrer des corrélations avec le risque cardiovasculaire dans des études prospectives mais son côté non invasive a offert la

possibilité d'étudier des changements dynamiques : après traitement antihypertenseur [75][76] ou antidiabétique [77].

1.3.2 Macrocirculation

1.3.2.1 Vitesse de l'onde de pouls

Les ondes voyagent plus vite dans des matériaux rigides, une perte de compliance artérielle résulte en une augmentation de la vitesse de propagation du pouls et une vitesse de l'onde de pouls est un marqueur d'artériosclérose. De très nombreuses techniques invasives et non invasives ont été décrites pour mesurer la rigidité artérielle. La plus utilisée est la mesure de l'onde de pouls carotido-fémorale (VOP-cf) et est considérée comme la méthode de référence [78] . La VOP-cf mesure le décalage entre les ondes de pouls au niveau carotidien droit et au niveau de l'artère fémorale droite mesuré « pied à pied » en utilisant un tonomètre d'applanation. Ce décalage est ensuite divisé par la distance entre les 2 sites multipliée par 0.8 pour normaliser la longueur du trajet artériel réelle. De nombreuses études ont permis d'établir des valeurs de référence et de montrer le rôle majeur de l'âge et de la pression sur la VOP-cf. Une valeur seuil de 10m/s a été établie comme reflétant une altération significative de la fonction aortique chez des hypertendus d'âge moyen.

1.3.2.2 Mesure des ondes de réflexion via l'index d'amplification

L'amplification de la pression artérielle résulte de mécanismes complexes. A chaque battement cardiaque, des ondes de pression et de flux sont générées et se propagent vers la périphérie où elles sont reflétées pour différentes raisons (gradient de rigidité, présence de bifurcations, modification de calibre au niveau des artéioles). A leur retour, ces ondes fusionnent avec les ondes antérogrades suivantes pour les amplifier. Il en résulte que les pressions périphériques sont supérieures aux pressions centrales, c'est l'amplification.[79] Avec l'âge et l'hypertension, la rigidification des parois augmente la rapidité du retour des ondes de réflexion qui arrivent en systole. Avec la vasoconstriction, l'amplitude des ondes de réflexion augmente. Tous ces phénomènes amènent à une augmentation de la pression systolique centrale. Les pressions centrales et les indices d'amplification (index d'amplification) peuvent être mesurés de façon invasive ou non invasive. L'analyse classique non invasive consiste en l'analyse isolée d'une onde de pression au niveau carotidien, brachial ou radial [20]. Ensuite, une fonction de transfert calibrée à partir de mesures invasives

permettra de déduire la forme de la courbe de pression centrale et donc les indices de pression et d'amplification : pression augmentée (augmentation de pression due aux réflexions) et index d'amplification (pression augmentée/pression pulsée). L'augmentation des ondes de réflexion a été étudiée dans de très larges populations montrant qu'elle est très fortement déterminée par le sexe, de l'âge et les facteurs de risque traditionnels et aussi qu'elle serait liée au risque cardiovasculaire chez les hommes [80].

1.3.2.3 Distensibilité aortique en IRM

La mesure de la VOP-cf, des pressions centrales et des ondes de réflexions donne accès à des paramètres de rigidité et de fonction aortiques globaux. L'IRM peut estimer la compliance de la paroi aortique soit 1) en mesurant les variations relatives de surface trans sectionnelle en coupe d'un segment choisi en utilisant l'écho de spin synchronisé sur l'ECG ou des séquences d'écho de gradient, soit 2) en mesurant la VOP à travers l'arche aortique en contraste de phase [81]. Des logiciels de segmentation semi automatisé validés [82] permettent ainsi de calculer : 1) le strain aortique = $(\text{Aire max} - \text{Aire min}) / \text{Aire min}$, et 2) la distensibilité aortique = strain / pression pulsée centrale. Cette méthode a permis de montrer une diminution de la distensibilité et du strain chez les hypertendus [83], de préciser les relations avec le vieillissement [84] et surtout de relier une diminution de distensibilité segmentaire au niveau de l'aorte ascendante à un risque cardiovasculaire augmenté [85].

1.3.2.4 Mesure de l'épaisseur intima média

L'infiltration de l'espace sous intimal peuvent être visualisées par plusieurs techniques si leur résolution spatiale est suffisante. Ainsi, l'épaisseur Intima Média (IMT) peut mesurée en mode B des ultrasons. Elle est mesurée comme la distance entre l'intima et l'adventice visualisée par un double liseré hyper/hypoéchogène. Selon le consensus de Mannheim [86], elle est plus facilement mesurée au niveau de la carotide commune. L'analyse est généralement faite par un logiciel de lecture semi automatisé sur un segment d'1cm, ou au mieux grâce à un système d'écho-tracking qui a permis la publication de valeurs de références [87] mais qui n'est pas disponible sur la majorité des appareils commercialisés. Une valeur élevée d'IMT ($>75^{\text{ème}}$ percentile [88] ou $>0.9\text{mm}$ [89]) est généralement acceptée comme témoignant de processus athéromateux plus globaux et d'un haut risque cardiovasculaire.

2 Hypothèses

2.1 Hypothèse n°1

Nous avons détaillé dans l'introduction les influences mêlées de l'âge et des facteurs de risque cardiovasculaires sur l'épaisseur artérielle ainsi que les interactions entre structure et fonction artérielle. L'âge et l'hypertension s'accompagnent d'une augmentation de la réflexion des ondes artérielles survenant sur les sites d'impédance différentes tout au long de l'arbre artériel. L'indice d'augmentation (Ai) est un index défini comme le rapport de la pression d'augmentation (qui est la différence de pression entre les pics de pression systolique précoce et tardif) sur la pression pulsée. Les déterminants de l'Ai sont les propriétés élastiques de l'aorte, l'éjection ventriculaire gauche ainsi que l'amplitude et le retard des ondes de réflexion artérielles. Il a été montré que l'Ai pouvait prédire les événements cliniques cardiovasculaires indépendamment de la pression sanguine périphérique et une des hypothèses mécanistiques avancées était que cette relation était médiée par une augmentation de la rigidité artérielle et de la pression pulsée pouvant conduire à une hypertrophie ventriculaire gauche et une diminution du flux sanguin coronaire. Cependant, la principale cause d'événements cardiovasculaires est constituée par l'athérosclérose et non pas les événements liés à l'augmentation de pression.

Notre première hypothèse était qu'il était possible de relier athérosclérose et rigidité artérielle (structure et fonction) en utilisant des moyens d'imagerie non invasifs que sont la mesure de l'intima média au niveau carotidien et la mesure des ondes de réflexion au niveau radial.

2.2 Hypothèse n°2

L'hypertension et l'âge affectent la structure des petites artères. Cependant nous avons vu dans l'introduction que les méthodes de références ne sont pas applicables en routine de par leur nature invasive. L'approche qui consiste à utiliser la rétine comme une fenêtre vers la microcirculation a été validée mais les techniques utilisées jusqu'à présent soient manquant de résolution, soit ne sont pas disponibles. En pratique clinique, l'angiographie ou le fond d'œil standard ne permettent pas l'évaluation fine des parois artérielles.

Notre hypothèse était qu'une nouvelle technique d'imagerie non invasive du fond d'œil : l'optique adaptative permettait d'analyser de façon précise les anomalies des artérioles rétinienne chez des patients atteints d'hypertension. En particulier, l'hypothèse était qu'au niveau des lésions focales causées par l'hypertension (les rétrécissements artériels focaux et les croisements artérioveineux) il n'existait pas d'augmentation de l'épaisseur pariétale mais une diminution de la lumière pouvant témoigner d'un remodelage « fonctionnel » et non structurel.

2.3 Hypothèse n°3

Les principaux facteurs hémodynamiques qui déterminent la pression artérielle sont le débit cardiaque et les résistances périphériques totales (TPR). Ces dernières reflètent le tonus vasculaire des petites artères. Comme nous l'avons vu dans l'introduction, le remodelage et donc la diminution de la lumière des artérioles peuvent donc jouer sur les TPR et la pression. Notre hypothèse était qu'il était possible d'établir une relation entre remodelage des petites artères et une mesure des TPR en utilisant des techniques d'imagerie non invasives de précision.

Par ailleurs, parallèlement aux changements microvasculaires, les altérations des grosses artères secondaires à l'hypertension ont été étudiées en profondeur en utilisant un large panel de méthodes invasives ou non invasives. Des études récentes ont indiqué qu'une combinaison de mesures centrales de pression avec CMR permettre une évaluation complète de l'aorte proximale à travers des indices fiables de géométrie et de distensibilité. Notre seconde hypothèse était qu'il existait des relations entre WLR et les grandes caractéristiques structurelles et fonctionnelles des grosses artères dans l'hypertension artérielle.

2.4 Hypothèse n°4

L'âge et l'hypertension s'accompagnent tous deux d'un remodelage micro vasculaire. Le concept physiopathologique le plus répandu est que cet épaissement pariétal est secondaire à une augmentation de la pression artérielle qui stimule la vasoconstriction myogénique afin de normaliser la tension pariétale. Par ailleurs, quelques études sur l'effet du contrôle de la pression artérielle sur la morphométrie des artérioles rétinienne ont été rapporté une amélioration à long terme du remodelage. Enfin plusieurs études ont montré que

certaines classes d'antihypertenseurs tels que les inhibiteurs du système rénine angiotensine possédaient un effet anti remodelage spécifique au-delà de la baisse tensionnelle par opposition à d'autres tels que les betabloquants qui bien que diminuant la pression ne permettaient pas un effet antiremodelage.

Notre hypothèse était qu'il était possible, grâce à l'optique adaptative d'étudier les effets relatifs de la pression artérielle de l'âge et des traitements antihypertenseurs sur les différents composants des artérioles rétinienne (épaisseur pariétale, diamètre luminal, surface trans-sectionnelle et indice de remodelage) dans une large population de sujets présentant des facteurs de risque et de l'hypertension.

3 Résultats

3.1 1^{er} article: Radial augmentation index is a surrogate marker of atherosclerotic burden in a primary prevention cohort

Le but de ce premier travail était d'étudier les liens entre les ondes de réflexions mesurées de façon non invasive au niveau d'une artère périphérique (l'artère radiale) et l'atteinte artérielle athéromateuse évaluée par son critère de substitution le plus validé : l'épaisseur de la carotide commune.

Pour ce faire nous avons étudié 1007 patients en prévention primaire qui présentaient au moins 1 facteur de risque traditionnel. Chez ces patients, l'index d'amplification au niveau de l'artère radiale a été mesuré de même que l'épaisseur intima média de la carotide commune (en dehors des zones présentant une plaque athéroscléreuse) et le risque cardiovasculaire global a été calculé en utilisant le score de Framingham à 10 ans.

Dans notre population âgée en moyenne de 56 ans et composée à 55% d'hommes nous avons retrouvé les différences précédemment décrites entre hommes et femmes. C'est-à-dire un Ai plus élevé chez les hommes que chez les femmes ($77 \pm 12\%$ contre $86 \pm 12\%$) ainsi qu'une relation entre l'âge et l'Ai différente avec l'obtention d'un plateau plus prononcé et plus rapide chez les femmes. Nous avons retrouvé les corrélations négatives connues entre l'Ai et la taille et le poids ainsi que les positives avec l'âge, la pression artérielle systolique, la pression pulsée, le diabète, le taux de HDL-C et la glycémie à jeun. Nous avons aussi retrouvé une corrélation positive entre Ai, épaisseur intima média, présence de plaque et risque cardiovasculaire (Figure 3 IMT chez les hommes en fonction des tertiles de risque Framingham (FRS) et d'augmentation radiale (rAI)).

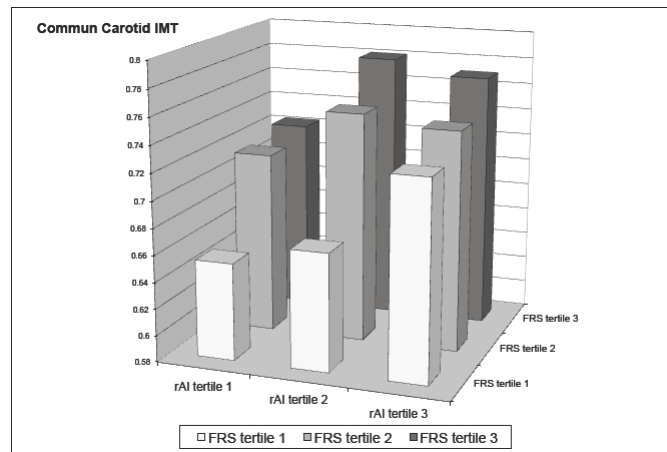


Figure 3 IMT chez les hommes en fonction des tertiles de risque Framingham (FRS) et d'augmentation radiale (rAI)

L'analyse multivariée effectuée chez les hommes et dans la population globale montrait que le lien entre Ai et épaissement de l'intima média carotidienne et entre Ai et présence de plaques était indépendante du risque cardiovasculaire (**Erreur ! Source du renvoi introuvable.**).

	Global population		Male		Female	
		<i>p</i>		<i>p</i>		<i>p</i>
Plaque presence	Chi2		Chi2		Chi2	
- Framingham CVRisk,	67.3	<i><0.0001</i>	25.5	<i><0.0001</i>	20.8	<i><0.0001</i>
- rAI	7.4	<i>0.007</i>	8	<i>0.005</i>	0.5	<i>0.47</i>
Max Carotid IMT	F		F		F	
- Framingham CVRisk,	67.3	<i><0.0001</i>	7.8	<i>0.005</i>	36.6	<i>0.0001</i>
- rAI	4	<i>0.047</i>	9	<i>0.003</i>	2.2	<i>0.14</i>

Table 1Analyse multivariée des déterminants de l'IMT et de la présence de plaques athéroscléreuses

Conclusion:

Il a été proposé que les liens entre rigidité et athérome puissent être expliqués par la pression pulsée. Notre hypothèse est que les ondes de réflexion puissent causer une augmentation du stress oscillatoire pariétal qui a été montré comme un facteur favorisant l'athérome. Ainsi nous suggérons que le potentiel de prédiction des événements cardiovasculaires par l'Ai ne passe pas uniquement par la pression pulsée et les événements liés à ses effets mais aussi par l'athérome dont nous avons montré qu'il est un reflet.

Ce travail a fait l'objet d'une publication dans Atherosclerosis en 2013.

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Radial augmentation index is a surrogate marker of atherosclerotic burden in a primary prevention cohort



David Rosenbaum^{a,b,*}, Philippe Giral^{a,b}, John Chapman^b, Fabiana H. Rached^{b,c}, Jean Francois Kahn^a, Eric Bruckert^{a,b}, Xavier Girerd^{a,b}

^a Unité de Prévention Cardiovasculaire, Service d'Endocrinologie-Métabolisme, Assistance Publique/Hôpitaux de Paris, Groupe Hospitalier Pitié-Salpêtrière, Université Pierre et Marie Curie, Paris, France

^b Dyslipoproteinemia and Atherosclerosis Research Unit, UMRS 939, National Institute for Health and Medical Research (INSERM) and Pierre et Marie Curie University (UPMC – Paris VI), Paris, France

^c Heart Institute-InCor, University of Sao Paulo, Sao Paulo, Brazil

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ABSTRACT

Arterial stiffness is linked to cardiovascular risk and predicts clinical events independently of peripheral blood pressure. The potential relationship between the augmentation index measured at the radial artery and asymptomatic atherosclerosis remains unclear however.

In order to assess relationship between the peripheral augmentation index and traditional risk factors, we estimated cardiovascular risk and presence of subclinical atherosclerosis in a large asymptomatic population in primary prevention.

Patients in primary prevention ($n = 1007$) with at least 1 cardiovascular risk factor were included and radial augmentation index was measured. Maximum common carotid intima-media thickness, the presence of plaque and Framingham 10 year cardiovascular risk score were assessed.

The mean augmentation index was $81 \pm 13\%$ in a population composed of 55% males (mean age 56 years). The augmentation index differed significantly between men ($77 \pm 12\%$) and women ($86 \pm 12\%$). In the global population, augmentation index was negatively correlated to height and weight, and positively correlated to cardiovascular risk, age, systolic blood pressure, pulse pressure, diabetes, HDL-Cholesterol, fasting glucose, intima-media thickness and to the presence of plaques.

Multivariate analysis in the global and in the male population revealed an independent and positive relationship between augmentation index and intima-media thickness on the one hand, and between augmentation index and the presence of plaque on the other.

Our results confirm that there are significant relationships between a surrogate marker of arterial stiffness and subclinical atherosclerosis in a large primary prevention population.

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1. Introduction

While hypertension is undoubtedly a major risk factor for the development of cardiovascular disease and premature atherosclerosis, nonetheless the potential clinical relevance of arterial wave reflection occurring at sites of impedance mismatch is still unclear.

The augmentation index (Ai) is an index indicative of backward traveling pressure waves, and is defined as the ratio of augmentation pressure (difference in pressure between the early and late

systolic shoulders) to pulse pressure [1]. The fundamental determinants of Ai are the elastic properties of the aorta, left ventricular ejection pattern and the amplitude and timing of arterial wave reflections.

A recent study [2] showed that central Ai predicts clinical events independently of peripheral blood pressure, possibly through an increase in arterial stiffness and in pulse pressure (PP), which may lead to left ventricular hypertrophy and decreased coronary blood flow. However, the major cause of cardiovascular (CV) events is not PP, but rather underlying atherosclerotic disease. Until now, the potential relationships between pressure wave reflections and atherosclerotic burden have not been evaluated in large unselected populations.

Several non-invasive techniques have been validated for the assessment of aortic and peripheral waveforms. First, carotid

* Corresponding author. Unité de Prévention Cardiovasculaire, Service d'Endocrinologie Métabolisme, Groupe Hospitalier Pitié-Salpêtrière, Assistance Publique Hôpitaux de Paris, 83, boulevard de l'Hôpital, 75651 Paris Cedex 13, France. Tel.: +33 1 42 17 57 76; fax: +33 1 42 17 78 32.

E-mail address: David.rosenbaum@psl.aphp.fr (D. Rosenbaum).

applanation tonometry can record carotid waveforms which are very close and similar to those in the aorta. Although direct measurements can be done [3], it can be challenging in some populations, even in experienced hands. The alternative approach is to perform radial artery tonometry and to use the radial waveform as a surrogate of the central waveform [4]. The radial artery pressure wave is composed of three waves: an incident wave generated by blood flow and two reflected waves, an initial one from the hand region and a subsequent wave arriving from the lower body. The radial artery augmentation index (rAI) is defined as the ratio between systolic peaks. Radial tonometry is simple, quick, well tolerated and new devices have recently been validated that enable central hemodynamic assessments in large populations [5,6].

The principal aim of our study was to assess the relationships between the peripheral rAI, and the presence of subclinical atherosclerosis in a large population of male and female patients in primary prevention.

2. Material and methods

2.1. Population

From March 2010 to July 2011, 1007 consecutive patients without a prior history of CV disease were included in this study at the Cardiovascular Prevention Unit of the Cardiology, Metabolism and Nutrition Institute (ICAN) at La Pitié Salpêtrière Hospital in Paris, France. The inclusion criteria consisted in having at least 1 CV risk factors among the following: hypertension, dyslipidemia, diabetes mellitus or current smoking. Arterial hypertension was considered present when measurement of brachial BP exceeded 140 mm Hg (systolic) and/or 90 mm Hg (diastolic) on at least two different occasions, or if the patient was on antihypertensive medication. Dyslipidemia was defined as a total serum cholesterol level of >5.0 mmol/L, serum LDL cholesterol level of >3.0 mmol/L, serum HDL cholesterol level of <1.0 mmol/L for men or <1.2 mmol/L for women, or serum triglycerides level of >1.7 mmol/L, or as a daily intake of any lipid-lowering medication. Diabetes mellitus was defined by fasting blood sugar levels ≥ 7.0 mmol/L or HbA_{1c} $> 6.5\%$. Additionally, the presence of diabetes mellitus was assumed if the patient was receiving any anti-diabetic treatment. Current smoking habits were divided into either current smoking (defined as any cigarette in the last month), or never smoked/smoking stopped.

Patients underwent complete medical interrogation and physical examination. After 12 h, fasting blood was sampled to ascertain levels of glucose, total cholesterol, triglycerides, LDL and HDL cholesterol, HbA_{1c} (for diabetic patients) and creatinine. During physical examination, bilateral brachial BP measurement was performed and patients were excluded if the inter arm BP difference was >10 mmHg for the systolic or the diastolic BP.

CV risk was assessed using the Framingham cardiovascular risk score (FRS) according to the Framingham equation [7].

The local institutional review board approved the study and informed consent was obtained from all the patients.

2.2. AI measurements

RAI measurements were made at a standard time in the morning between 10.30 and 11.30 am by the same trained operator in a temperature-controlled room where each subject rested supine for 5 min and refrained from smoking, heavy exercise, and consumption of alcohol or caffeinated beverages for at least 2 h before measurements were initiated.

Brachial BP was measured at the time of rAI measurement with the participant in a sitting position. Brachial BP was measured using

a validated automatic cuff oscillometric device. At least 4 brachial BP measurement were taken within a 10 min period until the difference between systolic BP values was <10 mmHg; the average of the last 2 BP readings was taken to determine the BP parameters included in the analysis. As rAI was systematically measured on the left wrist and in order to avoid perturbation of interpretation of the rAI, patients with an inter arm BP difference >10 mmHg were excluded. The radial pulse wave was recorded using automated applanation tonometry (HEM-9000AI; Omron healthcare). RAI measurements made with the OMRON HEM9000 AI instrument were validated against central catheterization which provided similar accuracy to those measured with the SphygmoCor® Device.

RAI was calculated as the ratio of the amplitude of the late systolic peak to the amplitude of the early systolic peak on the radial waveform. RAI values were determined for each pulse over a 30 s period and a mean rAI was calculated by the device for each patient and adjusted for a heart rate of 75 beats per minute. This final adjusted rAI was used for analyses. A preliminary study on a sample of our population (30 patients) showed a coefficient of variation of $2.7 \pm 2.6\%$ for 2 consecutive measurements.

2.3. IMT measurements and plaque assessment

Measurements were made over a 1 cm segment in the distal CCA (1 cm proximal to dilation of the carotid bulb) and over, 1 cm segment of the carotid artery bifurcation (1 cm proximal to the flow divider) on both right and left sides using an ACUSON Sequoia system (8 MHz transducer). Eleven far-wall IMT measures were obtained at 1 mm increments. In addition, information as to the presence or absence of plaque was also recorded. Trained readers using the presence or absence of two of the following three criteria judged the presence of plaque: abnormal wall thickness (defined as $IMT > 1.5$ mm), abnormal shape (protrusion into the lumen and loss of alignment with adjacent arterial wall boundary), and abnormal wall texture (brighter echoes than adjacent boundaries) [8]. For the purpose of this analysis, both right and left carotid arteries were assessed and information about the presence or absence of plaque in each subject were used. Maximum IMT was defined for each subject as the maximum IMT at a plaque free area in either the right or the left common carotid.

2.4. Statistics

Subject characteristics were expressed as means \pm SD or frequencies. Descriptive analysis was performed for clinical and anthropometric characteristics of each patient; continuous results are presented as mean \pm standard deviations [Table 1]. To compare patient characteristics, the *T*-Test was used for continuous and chi2 for dichotomous variables respectively [Table 1]. Univariate [Table 2] and multivariate [Table 3] regression analyses were performed to assess the determinants of rAI using various clinical variables. The variables that were statistically significant ($p < 0.05$) in the univariate analyses and non-redundant with the formula used in order to assess Framingham cardiovascular risk were then evaluated in the multivariate analysis for the assessment of determinants of IMT by multiple regression and plaque burden by multiple logistic regression [Table 3]. We also evaluated the relationships between RAI, maxIMT and FRS in 3 age subgroups: participant ≤ 50 years old, participants >50 and <70 and those ≥ 70 years.

RAI distribution in males and females was analyzed using a type 2 polynomial regression [Fig. 1].

All statistical analyses were performed using JMP statistical software. For all tests, $p < 0.05$ was considered significant. All

Table 1
Characteristics of the participants.

Characteristic	All (N = 1007)	Male (N = 559)	Female (N = 448)
Age – year	56.3 ± 11.8	57 ± 12.1	56.2 ± 11.6
Radial Augmentation index – %	81 ± 13	77 ± 12	86 ± 12†
Weight – kg	75.7 ± 15.8	81.6 ± 13.9	68.3 ± 14.8†
Height – cm	168 ± 9	173 ± 7	161 ± 7†
BMI – kg/m ²	26.8 ± 4.9	27.1 ± 4.0	26.5 ± 5.7*
Hypertension – no. (%)	569 (56.5)	336 (60.1)	233 (52)*
Blood pressure – mmHg			
Systolic	126.3 ± 15.9	128.0 ± 15.1	124.2 ± 16.7†
Diastolic	62.0 ± 10.5	74.1 ± 9.9	70.4 ± 10.9†
Mean	90.4 ± 11.3	92.0 ± 10.6	88.3 ± 11.8†
Pulse	53.9 ± 11.6	54.0 ± 11.2	53.7 ± 12
Diabetes mellitus – no. (%)	125 (12.4)	75 (13.4)	50 (11.2)
Fasting Glucose – mmol/L	5.37 ± 1.2	5.47 ± 1.14	5.25 ± 1.32†
Dyslipidemia – no. (%)	515 (51.1)	320 (57.2)	195 (43.5)†
Cholesterol – mmol/L			
Total	5.2 ± 1.2	5.2 ± 1.2	5.7 ± 1.3†
Low density lipoprotein	3.4 ± 1.2	3.3 ± 1.2	3.6 ± 1.2†
High density lipoprotein	1.3 ± 0.4	1.2 ± 0.4	1.6 ± 0.5†
Current cigarette smoking – no. (%)	175 (17.3)	111 (19.9)	64 (14.3)*
Cardiovascular risk – % at 10 years	9.7 ± 7.3	12.7 ± 0.3	5.9 ± 0.3†
maximum intima media thickness – mm.	0.70 ± 0.17	0.72 ± 0.18	0.67 ± 0.16†
Carotid plaque presence – no. (%)	566 (57%)	342 (61.6%)	224 (50.6%)†

*p < 0.05, †p < 0.01, ‡p < 0.0001 for difference between male and female.

statistical analyses were made separately in males, in females and in the total population.

3. Experimental results

3.1. Population characteristics

A total of 1007 patients were included in the study; their clinical and biological characteristics are shown in Table 1. The mean age of this population was 56.3 years; 55.5% of patients were men. The age of patients in our study ranged from 23 to 80 for males and 20–80 years for females.

Among the patients, 56.5% were treated for hypertension, 12.4% for diabetes mellitus, 51.1% for dyslipidemia, and 17.3% were current smokers.

In our population, several differences were evident between men and women. Men had higher BMI, were significantly taller and a greater proportion displayed presented hypertension, dyslipidemia and were current smokers. Also, males presented with thicker IMT and a higher incidence of carotid artery plaques.

Table 2
Univariate analysis of rAI determinants in male, female and in all participants.

	Global population R	Male R	Female R
Age	0.38†	0.41†	0.37†
Height	−0.42†	−0.28†	−0.23†
Framingham risk score	0.07*	0.35†	0.37†
Hypertension status	0.11†	0.16†	0.17†
Pulse pressure	0.09†	0.1*	0.08
Current smoking	0.01*	0.05	0.03
Diabetes	0.07*	0.17†	−0.02
Dyslipidemia	0.02	0.16*	−0.02
Maximum IMT	0.08†	0.18†	0.11*
Plaque presence	0.1†	0.21†	0.07

*p < 0.05, †p < 0.01, ‡p < 0.0001.

Table 3

Regression analysis of IMT max and Plaque presence determinants in the global population, in males and in females. Variables included in the regression were: Framingham risk score, rAI, height, weight, diastolic blood pressure, antihypertensive treatment and lipid lowering treatment.

	Global population	Male	Females
IMT max (R ²)	0.07†	0.05†	0.11†
Risk (β ± SD)	0.0061 ± 0.0007†	0.0029 ± 0.0011†	0.0093 ± 0.0015†
rAI (β ± SD)	0.0008 ± 0.0004*	0.0016 ± 0.0007*	0.0009 ± 0.0006
Plaque presence (R ²)	0.07†	0.07†	0.04†
Risk (β ± SD)	−0.0924 ± 0.0113†	−0.0787 ± 0.0157†	−0.1139 ± 0.0248†
rAI (β ± SD)	−0.0139 ± 0.0051†	−0.0238 ± 0.008†	−0.0058 ± 0.0083

*p < 0.05, †p < 0.01, ‡p < 0.0001.

3.2. rAI distribution

Mean rAI for the total cohort was 81 ± 13% but differed significantly between men (77% ± 12) and women (86% ± 12). rAI increased with age with a plateau at about the age of 56 years for males and 57 years for females as revealed by the polynomial data fit (Fig. 1).

3.3. Univariate analysis of rAI determinants (Table 2)

In the global population, rAI was correlated to age, total cardiovascular risk, hypertension, systolic blood pressure, pulse pressure, diabetes, HDL-C and fasting glucose. Moreover, rAI was especially strongly and negatively correlated to height and weight. rAI was correlated to the right common carotid IMT, to the maximal IMT and to the presence of plaque.

As we found significant differences between males and females in our population, we evaluated the potential relationships of rAI with traditional risk factors in each gender separately.

In males, rAI was correlated to diastolic blood pressure, mean blood pressure, dyslipidemia and fasting glucose level. In females, rAI was correlated to systolic blood pressure, mean blood pressure, LDL-C and HDL-C.

Furthermore, both left and right IMT values as well as maximal IMT values were correlated to rAI in both genders. By contrast, the presence of plaque was correlated to rAI in men only.

3.4. Multivariate analysis of IMT and determinants of atherosclerotic burden (Table 3)

In the global population, a multivariate analysis including age, sex, height, weight, total cholesterol, HDL cholesterol, systolic blood pressure, diastolic blood pressure, antihypertensive treatment, lipid lowering treatment, smoking status and diabetic status identified an independent association between rAI (p < 0.01) and elevated IMT (p < 0.01), and between rAI and presence of plaque. As Framingham risk score may underestimate the contribution of each risk factor, we also evaluated those relationships in a multivariate model. In this model, we obtained similar findings as those given by the Framingham risk score (data not shown).

As seen in Fig. 2, IMT elevation in males increased progressively both with rAI tertiles and especially with tertiles of FRS. The highest IMT was found in males who presented the highest rAI and the highest FRS.

When analyzed separately, the independent relation between rAI and presence of plaque and IMT were confirmed in males but not in females. Because of the flat relationship between rAI and age in females above 57 years, we analyzed a subgroup of 199 females aged 20–56 years. Although a linear relation was found between age and rAI, there were no links between rAI, IMT and plaque presence in multivariate analysis in this subgroup (data not shown).

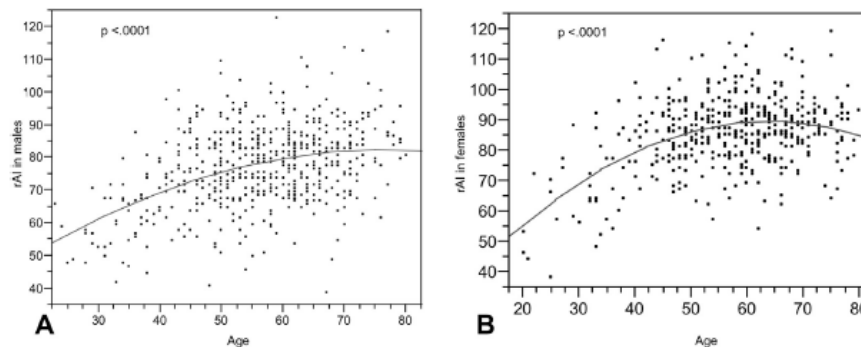


Fig. 1. rAI distribution in male and female according to age (type 2 polynomial regression).

Subgroup regression analysis on the basis of age showed that maxIMT mainly correlated to rAI in men from 50 to 70 years of age.

4. Discussion

In a large population of patients exhibiting a wide range of risk factors in primary prevention, we observed that peripheral rAI correlated with several CV risk factors (age, hypertension, diabetes, dyslipidemia) and with global CV risk as previously established. Moreover, our data indicate that rAI is a significant determinant of atherosclerotic burden independently of global CV risk and individual risk factors, thereby suggesting a possible link between the functional properties of the arterial wall and asymptomatic atherosclerosis itself. In addition, our gender-individualized data reveal limitations of the application of rAI in females. In the subgroup analysis on the base of age, we found that this correlation was the strongest in middle aged men, who are the main target of primary cardiovascular prevention intervention.

Our results are consistent with earlier studies that demonstrated the influence of age and height on rAI [9]. Previous reports have shown that carotid AI was linked to age, gender, height; diastolic blood pressure and heart rate [10–12] as well as to CV risk as assessed with 3 different risk algorithms [13]. Furthermore, our findings are consistent with earlier reports showing the same

association using radial tonometry AI [14]. We were also able to demonstrate the influence of hypertension and diabetes on centrally- [13,15] or peripherally-measured [14,16] AI.

Data from previous studies on the relationship between rAI and dyslipidemia are controversial, as both negative [13,17] and positive [18] associations have been reported. Our results revealed an association specifically in men. In earlier studies, no gender-individualized data were described. As for current smoking status, we were unable to detect an independent influence of smoking on radial AI as others have reported earlier [18]. A possible explanation for this discrepancy concerns the plateau reached by radial AI at the age of 56/57, and which is very close to the mean age of our population [19]. A similar hypothesis may hold for the lack of relation of rAI with dyslipidemia or diabetes in women.

Our study equally reveals strong relationships between radial AI and Framingham risk score, and therefore is coherent with earlier reports linking central AI and various cardiovascular risk scores (ESC, SMART and EPOZ) [13] or rAI and the MEGA risk score [17]. Our present findings, in addition to those of Duprez et al. in 300 asymptomatic young American patients at low risk, extend these observations [20].

RAI and carotid AI measured with different devices have already been shown to be associated with arterial remodeling and increase in IMT in selected populations [20–23]. By comparison, our findings are derived from a large population in primary prevention and were adjusted for gender together with other confounding risk factors.

Central AI has also been related to cardiovascular mortality in several populations [2] and a recent meta-analysis showed it to be an independent predictor of cardiovascular events and all-cause mortality [6]. As PWV is known to be a key determinant of AI, and as several studies have documented a link between increased cardiovascular risk and PWV, it has been postulated that the missing link between elevated AI and an elevated rate of cardiovascular events may derive from increased arterial stiffness and pulse pressure. This hypothesis proposes that low distensibility generates increased PP, thereby inducing decrease in diastolic coronary perfusion, together with increase in left ventricular hypertrophy and other target organ damage [15,24].

Two small studies on selected patients have already associated increased AI with biomarkers of atherosclerosis [25,26]. It is however the first time that a link between radial AI and atherosclerotic burden, independently of cardiovascular risk factors, has been reported in a large population in primary prevention.

The present findings suggest that wave reflections may be partly responsible for the increased cardiovascular risk in favoring the

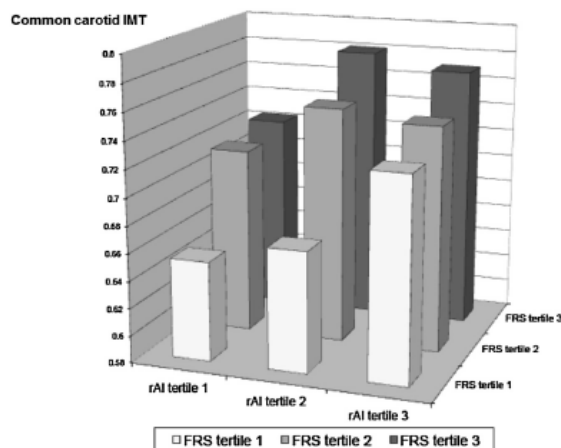


Fig. 2. IMT in male according to rAI tertiles and FRS tertiles.

development of premature atherosclerosis and may represent a link between AI and cardiovascular events. Beyond increase in pulse pressure, we hypothesize that wave reflections may cause an increase in wall oscillating shear stress; importantly, the latter has been shown to favor the progression of atherosclerosis [27]. Moreover, Beausseier et al. [28] recently demonstrated that plaques were the location of inward bending strain, which may in turn enhance plaque growth and rupture via stress induced cell signaling [29] and/or mechanical fatigue. AI is usually seen as a pure marker of pressure wave reflection and aortic stiffness. However recent findings in young adults [30] reported that central AI may be substantially determined by left ventricular systolic function. Along with this report, the fact that rAI could be an independent determinant of atherosclerosis suggests that it could be more than merely a haemodynamic marker.

Finally, our data have identified differences between the characteristics of rAI in males versus females. Indeed, in the multivariate analysis, plaque burden was not correlated to rAI in women. A possible explanation might be the plateau attained by rAI around the age of 60. In this regard it is relevant that elastic artery stiffness is lower in younger premenopausal women than in men. The changes in elastic artery stiffness paralleled those in brachial and carotid artery pulse pressures, which were lower in younger and higher in older women respectively as compared with their counterparts in men. However, rAI also reach a plateau in men. Another possible explanation is the potential deleterious role of androgens. Though they have not been thoroughly studied, androgen receptors are present in animal vascular tissues and male sex steroids may promote arterial stiffening and atherosclerosis by increasing smooth muscle cell proliferation and monocyte adhesion to endothelial cells. Furthermore observations that women display stiffer large arteries than men in the postmenopausal years (when sex steroid levels are relatively low) and that stiffer large arteries and higher pulse pressure are observed in prepubertal females also suggest that intrinsic gender differences may exist. In our study, 332 (74%) of the women aged more than 50 years and the differences in AI between pre- and postmenopausal women persisted after adjustment for various hormonal substitution therapies (data not shown), thereby corroborating the results from a recent prospective study in which rAI predicted mortality in males but not in women [31].

Our study exhibits some limitations that deserve to be mentioned. Firstly, its cross-sectional design does not allow investigation of the relationship between rAI and primary outcomes. Also, our population is not homogenous and includes a wide range of subjects treated with antihypertensive and lipid lowering medications. These risk factors and their treatment may have influenced our results and constitute significant cofounders. On the other hand, rAI could be seen as an integrator of the various effects of the risk factors on the arterial wall despite the prescribed medications. Heart rate is known to greatly influence rAI; however in this instance, the value obtained after adjustment for 75 bpm was employed in the analysis. Another limitation of our study is the lack measurement of central blood pressure. It could certainly have provided further insight into our findings especially if central systolic BP could have been included in the multivariate analysis. However, rAI and central systolic blood pressure are strongly correlated so including it in the analysis may not have modified our results. Another limitation of our study is the lack measurement of central blood pressure which would certainly have provided further insight into our findings especially if central systolic BP could have been included in the multivariate analysis. However, rAI and central systolic blood pressure are strongly correlated so the inclusion of this parameter including it in the analysis may not have modified our results. Another possible limitation is the exclusion of

patients with an inter-arm BP difference as those patients may display subclinical atherosclerosis. Furthermore, rAI measurements were performed only 2 h after consumption of caffeinated beverages which may have led to increase in rAI according to published studies. However, the influence of coffee consumption on rAI is mild (+5 to +7%) and is mainly explained by heart rate increase. We used adjusted rAI for heart rate; it greatly diminishes the possibility that our findings may have been influenced by this factor. In addition, the use of rAI as a surrogate for aortic AI can also be challenged but the device we used has been validated. Moreover, this device is rapid, easy and reliable to use in standard conditions, thereby facilitating the measurement of rAI in large populations.

Finally, this study on more than 1000 subjects in primary prevention with a wide distribution of risk factors sheds new light on the relationships between gender, wave reflections and early, subclinical atherosclerosis.

Recent technologic developments have enabled a large panel of arterial stiffness measures to be done with the most advanced devices. There is vast evidence that large and small arteries stiffening, as assumed functionally or measured structurally, is associated with a higher CV risk but direct comparisons between methods, devices and segment studied are sparse. The clinical utility of many of those measures remains unknown whereas the identification of a stiffening process that accelerates aging, promotes hypertension and may trigger atherosclerosis has stressed a need for intervention and monitoring. Our study reinforces the status of augmentation index as an independent CV risk marker by validating its measurement at the radial artery. Therefore, it is also one of the early steps in establishing the clinical utility of arterial stiffness assessments.

5. Conclusion

By revealing that a marker of arterial stiffness such as rAI is a significant determinant of atherosclerotic burden, our results confirm that there are significant relationships between arterial system biomechanics and subclinical atherosclerosis in a large primary prevention population. This observation equally suggests that the prediction of CV events by rAI may not solely be explained by pulse pressure determinants but to some degree by the presence of atherosclerotic lesions. Our study equally suggests that rAI could potentially serve as a rapid and simple surrogate marker of carotid atherosclerosis in routine clinical practice. Finally, we show that rAI assessment in clinical practice would provide valuable information in males.

Acknowledgment

None

List of abbreviations

AI	augmentation index
BP	blood pressure
CCA	common carotid artery
CV	cardiovascular
FRS	Framingham risk score
HDL	high density lipoprotein
IMT	intima media thickness
LDL	low density lipoprotein
PP	pulse pressure
PWV	pulse wave velocity
RAI	radial augmentation index

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Conflicts of interest

None.

Disclosure

None.

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3.2 2^{ème} article: Morphometric analysis of small arteries in the human retina using adaptive optics imaging: relationship with blood pressure and focal vascular changes

Le but de cette étude était de démontrer qu'il était possible d'obtenir une imagerie de la paroi des artérioles rétinienne de précision et de façon non invasive en utilisant une nouvelle technique d'imagerie par optique adaptative. Nous nous sommes aussi intéressés aux zones de rétrécissements focaux artériels afin de déterminer l'épaisseur du mur artériel à ces endroits.

Dans une cohorte de 49 patients hypertendus jamais traités, des images de fond d'œil par optique adaptative ont permis de mettre en évidence un remodelage eutrophique artériolaire (en dehors des zones de rétrécissement focaux), c'est-à-dire une augmentation du rapport mur/lumière (WLR) résultant d'une augmentation de l'épaisseur pariétale et d'une diminution de la lumière sans changement de la surface Trans-sectionnelle du vaisseau (Table 2 Caractéristiques cliniques et morphologiques de la population) en comparaison avec 30 normotendus. En analyse multivariée, le WLR était corrélé à l'âge et à la pression artérielle moyenne.

	Total	Normotensive	Hypertensive	P*
n	49 (23F, 26M)	30 (15F, 15M)	19 (8F, 11M)	
Age (years)	44.9 ± 14.4	42.3 ± 15	48 ± 11	NS
BMI	24.9 ± 4.7	23.8 ± 4.5	26.4 ± 4	NS
SBP (mmHg)	132.5 ± 22.2	118 ± 13	154 ± 14	<0.01
DBP (mmHg)	82.6 ± 14	74 ± 9.5	95.5 ± 10	<0.01
Mean BP (mmHg)	99 ± 16	88.8 ± 10	113.8 ± 11	<0.01
Pulse BP (mmHg)	49.9 ± 12	43.7 ± 9	58.9 ± 11	<0.01
D (μm)	79.8 ± 12	83.5 ± 11.2	74 ± 12.6	<0.05
P (μm)	24.3 ± 3.7	23.5 ± 3.7	25.5 ± 3.3	NS
WLR	0.31 ± 0.07	0.285 ± 0.05	0.36 ± 0.08	<0.01
WCSA (μm ²)	3411 ± 874	3459 ± 915	3338 ± 826	NS

BP, blood pressure; D, diameter; NS, not statistically significant; P, parietal thickness; WCSA, wall cross-sectional surface; WLR, wall-to-lumen ratio.
*Between normo and hypertensive.

Table 2 Caractéristiques cliniques et morphologiques de la population

Plus spécifiquement, aux endroits présentant un rétrécissement focal ou un croisement artérioveineux, l'optique adaptative a permis de mettre en évidence un mur artériel d'épaisseur inchangée par rapport aux sections artérielles saines contiguës. A la place, en cas de rétrécissement artériel, c'est une diminution des diamètres internes et externes des vaisseaux qui a été constaté sans modification de la surface trans-sectionnelle du vaisseau (**Erreur ! Source du renvoi introuvable.**).

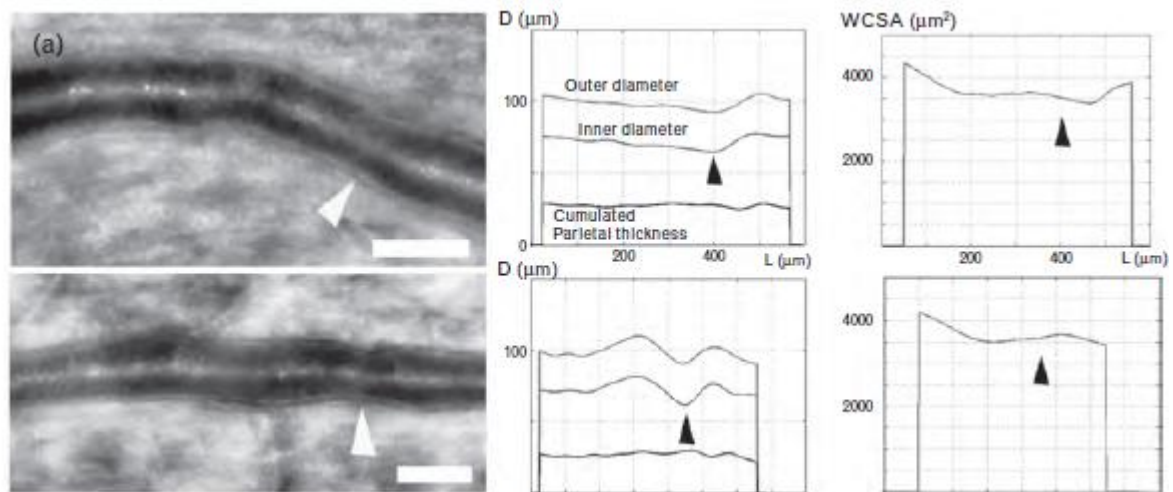


Figure 4 Imagerie de 2 cas de rétrécissement artériolaire focal (a) avec la mesure des indices anatomiques respectifs montrant le maintien du parallélisme des parois externes et internes sans changement de surface artériolaire (WCSA)

Conclusion :

Ce travail montre que l'optique adaptative est un outil d'imagerie non invasif de la microcirculation permettant l'analyse fine des altérations pariétales microvasculaires dans l'hypertension. La visualisation d'une épaisseur pariétale inchangée à l'endroit de rétrécissements focaux suggère que ceux-ci sont le fruit d'un remodelage fonctionnel impliquant des mécanismes de vasoconstriction.

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Morphometric analysis of small arteries in the human retina using adaptive optics imaging: relationship with blood pressure and focal vascular changes

Edouard Koch^a, David Rosenbaum^b, Aurélie Brolly^a, José-Alain Sahel^a, Philippe Chaumet-Riffaud^{a,c}, Xavier Girerd^b, Florence Rossant^d, and Michel Paques^a

Objectives: The wall-to-lumen ratio (WLR) of retinal arteries is a recognized surrogate of end-organ damage due to aging and/or arterial hypertension. However, parietal morphometry remains difficult to assess *in vivo*. Recently, it was shown that adaptive optics retinal imaging can resolve parietal structures of retinal arterioles in humans *in vivo*. Here, using adaptive optics retinal imaging, we investigated the variations of parietal thickness of small retinal arteries with blood pressure and focal vascular damage.

Methods: Adaptive optics imaging of the superotemporal retinal artery was done in 49 treatment-naïve individuals [mean age (\pm SD) 44.9 years (\pm 14); mean systolic pressure 132 mmHg (\pm 22)]. Semi-automated segmentation allowed extracting parietal thickness and lumen diameter. In a distinct cohort, adaptive optics images of arteriovenous nicking (AVN; $n=12$) and focal arteriolar narrowing (FAN; $n=10$) were also analyzed qualitatively and quantitatively.

Results: In the cohort of treatment-naïve individuals, by multiple regression taking into account age, body mass index, mean, systolic, diastolic and pulse blood pressure, the WLR was found positively correlated to mean blood pressure and age which in combination accounted for 43% of the variability of WLR. In the cohort of patients with focal vascular damage, neither FANs or AVNs showed evidence of parietal growth; instead, at sites of FANs, decreased outer diameter suggestive of vasoconstriction was consistently found, while at sites of AVNs venous narrowing could be seen in the absence of arteriovenous contact.

Conclusion: High resolution imaging of retinal vessels by adaptive optics allows quantitative microvascular phenotyping, which may contribute to a better understanding and management of hypertensive retinopathy.

Keywords: adaptive optics, arterial hypertension, retina, small vessels, wall-to-lumen ratio

Abbreviations: AO, adaptive optics; AVN, arteriovenous nicking; FAN, focal arteriolar narrowing; SDF, scanning laser Doppler flowmetry; WCSA, wall cross-sectional area; WLR, wall-to-lumen ratio

INTRODUCTION

Arterial hypertension and aging affects the structure of small arteries. An increase of the wall-to-lumen ratio (WLR) is a hallmark of hypertensive microangiopathy and is predictive of end-organ damage [1–4]. The prevalent physiopathological concept of such parietal thickening postulates that a rise in blood pressure stimulates myogenic vasoconstriction, which tends to normalize parietal tension [5,6], without significant modification of the parietal components (a process called eutrophic remodeling). However, there is currently a lack of clinically pertinent methods for measuring parietal thickness. Myographic and histological investigations are indeed not applicable in clinical routine. Conversely, the retina being an easily accessible part of the microcirculation, *in-vivo* evaluation of the microvascular consequences of arterial hypertension can be done on fundus photographs. The most prevalent lesions of hypertensive retinopathy are diffuse narrowing of arterioles and focal lesions such as focal arteriolar narrowing (FAN) and arteriovenous nicking (AVN). Several large-scale epidemiological studies reported that the severity and/or incidence of these signs correlate with past and incident arterial pressure [7–10] and with end-organ damage [11–14]. The clinical evaluation of hypertensive retinopathy is however limited by the fact that fundus photographs or fluorescein angiography do not enable visualizing the arteriolar wall. An indirect measure of the arteriolar wall thickness based on the differential analysis of

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^aClinical Investigation Center 503, Centre Hospitalier National des Quinze-Vingts, INSERM and Université Pierre et Marie Curie-Paris6, ^bPreventive Cardiovascular Unit, Institute of Cardiomatobiology and Nutrition, Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris, ^cUniversité Paris-Sud, Hôpital Bicêtre, Assistance Publique-Hôpitaux de Paris, and ^dInstitut Supérieur d'Electronique de Paris, Paris, France

Correspondence to Michel Paques, MD, PhD, Clinical Investigation Center 503, Centre Hospitalier National des Quinze-Vingts, 28 rue de Charenton 75012 Paris, France. Tel: +33 1 40 02 14 15; fax: +33 1 40 02 14 99; e-mail: michel.paques@gmail.com

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laser Doppler and reflectance imaging (scanning laser Doppler flowmetry, SLDL) of the retina has been proposed [15–17]. However, this technique has a relatively limited spatial resolution, which impairs in particular the analysis of focal lesions.

Adaptive optics is an opto-electronic technology that improves the resolution of fundus images. Current adaptive optics-based fundus cameras enable visualization of microstructures such as photoreceptors [18], capillaries [19] or vascular wall [20] noninvasively in humans. Here, following our pilot study [21], we evaluated a novel approach of microvascular morphometry using adaptive optics imaging, which may ultimately help to better understand and manage hypertensive microangiopathy.

METHODS

This clinical study was carried out according to the principles outlined in the Declaration of Helsinki. Approval of the Ethics Committee of the Saint-Antoine hospital (Paris, France) was obtained. Patients older than 18 years with clear ocular media and no ocular or systemic diseases apart from arterial hypertension were considered eligible. Patients were recruited at the Preventive Cardiovascular Unit of the Pitié-Salpêtrière Hospital. Other patients with AVNs and/or FANs were also recruited at the Quinze-Vingts Hospital. Each patient received full oral and written information and gave written consent prior to inclusion.

Adaptive optics retinal imaging

Retinal imaging was performed at the Clinical Investigation Center of the Quinze-Vingts Hospital. En face adaptive optics fundus images were obtained using a commercially available flood-illumination adaptive optics retinal camera (rtx1; Imagine Eyes, Orsay, France). Briefly, the rtx1 camera measures and corrects wavefront aberrations with a 750 nm super luminescent diode source and an adaptive optics system operating in a closed loop. A 4×4 fundus area (i.e. approximately 1.2×1.2 mm in emmetropic eyes) is illuminated at 840 nm by a temporally low coherent light-emitting diode flashed flood source, and a stack of 40 fundus images is acquired in 4 s by a charge-coupled device camera.

Most examinations were done without pupil dilation; if needed, pupil dilation was obtained with topical tropicamide (Novartis, France). After a 10-min rest during which the examination was explained, the patient was installed on the chin rest. The measured refraction was integrated into the camera. The live video image of the pupil allowed alignment with the incident light; the live display of adaptive optics-corrected fundus image allowed brightness, contrast and focus to be adjusted. Gaze was oriented by an internal or external target in order to capture the region of interest. The reference site was a segment of the supertemporal artery of the right eye, at least 250 μ m long with an inner diameter of at least 50 μ m, devoid of bifurcations, one disc diameter from the disc (see example in Fig. 1a). Blood pressure (BP) was measured in the sitting position simultaneously to adaptive optics image acquisition using an automated oscillometric device using an arm cuff (VS800, Mindray Corporation; Shenzhen, China). Two

measures of BP were taken before starting the acquisition process; then one BP measurement was then performed during each image acquisition.

To identify the systolic pulse and delete the corresponding images, real-time videos were generated from each stack using a customized plugin developed under ImageJ software (see examples in supplementary video 1 and 2, <http://links.lww.com/HJH/A317>, <http://links.lww.com/HJH/A318> [adaptive optics videofunduscopy of the supertemporal artery and vein in the right eye of a 26 years old man (same patient as in figure 1). Note the visibility of the arterial wall but not of the venous wall, and the systolic curving of the arteriole (Quicktime video; image width 1.2 mm; 40 frames, 10 fps); adaptive optics videofunduscopy of the supertemporal artery in the right eye of a 40 years old healthy woman (Quicktime video; image width 1.2 mm; 40 frames, 10 fps). Then, the diastolic images were averaged to increase the signal-to-noise ratio (Fig. 1b; see ref.[22] for supplementary details, <http://links.lww.com/HJH/A316>).

Image analysis

Averaged adaptive optics images were semi-automatically segmented using a custom software running under Matlab (Mathworks, Natick, Massachusetts, USA). Briefly, the processed adaptive optics images, after being encoded on 8 bits, were first enhanced by applying a median filter followed by a nonlinear diffusion filter [23]. Such filters allow smoothing the blood vessels while preserving the contrast along their edges. The first step of the segmentation is based on the enhancement of the axial reflection and the detection of the darkest regions, by applying respectively morphological operations and k-means classification. Both are then fused in order to select the axial reflection of the vessel and compute a binary mask of the vessel. The second step of the segmentation process aims at extracting the borders of the vessel. Each side is approximated by a curve parallel to the regularized skeleton of its axial reflection [24,25]. The mean distance between a side contour and the central reflection line is deduced from the binary mask and the gradient image; it is adjusted so that the obtained curve is placed as near as possible to the internal side of the parietal structure. This segmentation is then refined by applying a parametric active contour with a parallelism constraint [26]. In this model, a curve evolves towards the higher gradients of the image (the edges) while maintaining locally an approximate parallelism with the reference line (the axial reflection), which improves robustness regarding image noise. The algorithm is applied twice in order to segment the internal limits of the vessel lumen. Then, the initialization is automatically modified in order to segment the outer limits. Thus, a complete segmentation of the arterial wall is obtained (Fig. 1b), with a point-by-point correspondence between opposite sides of the vessel. The whole segmentation process is under human supervision. Graphic representations of morphometric parameters along a given vessel segment (termed here morphograms; Fig. 1c) were generated. The ratio of total parietal thickness (P) over the lumen diameter (D) averaged over 250 μ m defined the WLR. The cross-sectional surface of the vessel wall, averaged over 250 μ m, defined the wall cross-sectional area (WCSA). All measures were done in a masked fashion.

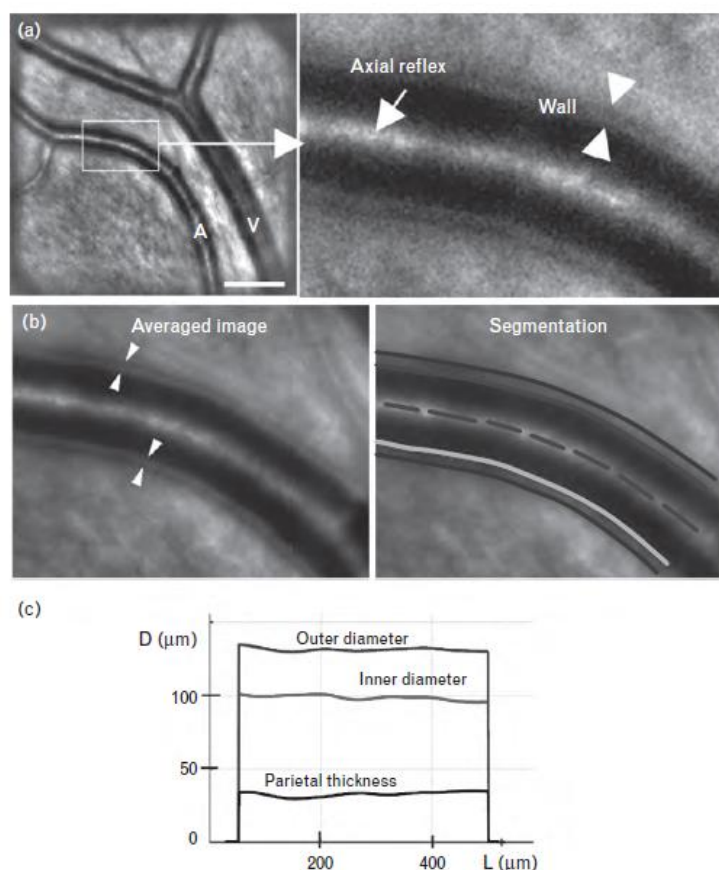


FIGURE 1 Adaptive optics (AO) imaging and segmentation of a retinal arteriole (same patient as in supplementary video 1, <http://links.lww.com/HJH/A317>). (a) Single video frame (right panel: magnification). Note that parietal structures (between arrowheads in magnification) can be seen (A: arteriole, V: venule; bar, 250 μ m). (b) Image averaging and segmentation. (c) Morphogram of the segmented vessel (D: diameter; L: length).

Statistics

Descriptive statistics of quantitative and ordinal variables and analysis of normality of distribution were performed. The means of quantitative variables between two groups were compared using the parametric *t* test for independent samples. The homogeneity of variance was checked using Levene's test. The relationship between two variables (interval data) was investigated by calculating Pearson's correlation coefficient. In some cases, Kendall's correlation values were also calculated. To test intra-observer and inter-observer reproducibility, three consecutive measures of WLR and lumen diameter were performed within 10 min in 20 patients, followed by a fourth measure at the same location 6 h later. Intraclass coefficients were over 0.8 and Cronbach's alpha were over 0.9 for lumen diameter and WLR (see supplementary Table 1 Table 1, <http://links.lww.com/HJH/A316>). The threshold of significance was set to $P=0.05$ for all tests. Pearson's correlation coefficient was calculated to estimate the linear relationship between two variables. Multiple regression (backward stepwise method) was carried out to identify predictors of WLR. All analyses

were performed with SPSS software (version 19; IBM Corporation, Arbank, New York, USA).

RESULTS

By adaptive optics imaging, the red cell column of arteries and veins appeared as dark stripes with an axial reflection. Along both sides of the blood column of arteries, a linear structure was visible (Fig. 1a). This structure was observed on individual video frames (supplementary videos 1 and 2, <http://links.lww.com/HJH/A317>, <http://links.lww.com/HJH/A318>) [adaptive optics video funduscopy of the superotemporal artery and vein in the right eye of a 26-year-old man (same patient as in figure 1). Note the visibility of the arterial wall but not of the venous wall, and the systolic curving of the arteriole (Quicktime video; image width 1.2 mm; 40 frames, 10 fps); adaptive optics video funduscopy of the superotemporal artery in the right eye of a 40-year-old healthy woman (Quicktime video; image width 1.2 mm; 40 frames, 10 fps)], hence ruling out a blurring artifact due to systolic expansion. Parietal

TABLE 1. Clinical and morphometric characteristics of the study population (mean \pm SD)

	Total	Normotensive	Hypertensive	P*
n	49 (23F, 26M)	30 (15F, 15M)	19 (8F, 11M)	
Age (years)	44.9 \pm 14.4	42.3 \pm 15	48 \pm 11	NS
BMI	24.9 \pm 4.7	23.8 \pm 4.5	26.4 \pm 4	NS
SBP (mmHg)	132.5 \pm 22.2	118 \pm 13	154 \pm 14	<0.01
DBP (mmHg)	82.6 \pm 14	74 \pm 9.5	95.5 \pm 10	<0.01
Mean BP (mmHg)	99 \pm 16	88.8 \pm 10	113.8 \pm 11	<0.01
Pulse BP (mmHg)	49.9 \pm 12	43.7 \pm 9	58.9 \pm 11	<0.01
D (μ m)	79.8 \pm 12	83.5 \pm 11.2	74 \pm 12.6	<0.05
P (μ m)	24.3 \pm 3.7	23.5 \pm 3.7	25.5 \pm 3.3	NS
WLR	0.31 \pm 0.07	0.285 \pm 0.05	0.36 \pm 0.08	<0.01
WCSA (μ m ²)	3411 \pm 874	3459 \pm 915	3338 \pm 826	NS

BP, blood pressure; D, diameter; NS, not statistically significant; P, parietal thickness; WCSA, wall cross-sectional surface; WLR, wall-to-lumen ratio.
*Between normo and hypertensive.

structures were visible in arterioles as small as 25 μ m. Their visibility did not depend on its orientation relative to the nerve fiber layer, ruling out an optical effect of ganglion cell axons.

Correlation of arteriolar morphometry and blood pressure

Forty-nine normotensive or treatment-naïve hypertensive individuals were included (Table 1). Nineteen had systolic pressure over 139 mmHg, while 30 were below. In hypertensive patients, the lumen diameter of the superotemporal artery was significantly lower, and the WLR was significantly higher. There was no significant difference of WCSA between groups. By univariate analysis, a number of significant correlations were found (Fig. 2 and Table 2). Multiple regression was carried out taking into account age, BMI, systolic, mean, and pulse pressure. The linear combination of these factors that gave the most accurate prediction of WLR was:

$$\text{WLR} = 0.0051 + 0.0025 \\ \times \text{mean pressure} + 0.0014 \times \text{age}$$

which accounted for 43% of the variability of WLR. This suggests that mean pressure had a stronger effect on WLR than age.

Adaptive optics imaging of focal vascular changes

In a distinct cohort, adaptive optics images of arteriovenous crossings and FANs were analyzed. Classical concepts of the pathophysiology of AVNs postulate that venous nicking is due to mechanical compression from overlying arterioles. Alternatively, the implication of retinal cells was suggested by histology. In order to progress in the understanding of AVNs, adaptive optics images of 10 normal arteriovenous crossings from nine patients (age range, 26–62 years) were compared with adaptive optics images of 12 AVNs from 12 patients (age range, 47–77 years). In normal arteriovenous crossings (Fig. 3a), venules were seen crossing under the arteriole without notably changing their caliber or their pathway. The borders of the vein lumen remained clearly visible up to the area of arteriovenous overlap. In AVNs (Fig. 3b and c, and supplementary Figure 1, <http://links.lww.com/HJH/A316>), the vein appeared frequently blurred

upstream and downstream. One or more sites of focal venous narrowing could be often seen upstream and/or downstream of the crossing site (asterisks in figures). The overlying arteriole did not show evidence of parietal thickening; The WLR was indeed not significantly different between AVNs and control areas (Fig. 3b). In order to better understand the arteriovenous relationship at sites of AVNs, we documented by adaptive optics four cases of venous nicking occurring at a site where an arteriole and a venule ran in parallel, yet without overlapping (Fig. 4c and supplementary Figure 2, bottom, <http://links.lww.com/HJH/A316>). This peculiar anatomical feature, which is clinically and histologically similar to AVNs with overlapping vessels [19] allowed a direct observation of the arteriovenous interface. In all cases, there was a gap 10–30 μ m wide between the artery and the vein, suggesting that physical contact between the arteriole and the venule is not a prerequisite for venous nicking.

In 10 FANs from 10 patients (age range, 47–64 years), we observed that, in most cases, the inner and outer vascular limits remained parallel throughout the FAN (Fig. 4 and supplementary Figure 2, <http://links.lww.com/HJH/A316>), focal parietal thickening being detected in only two cases. Therefore, the WLR in FANs was locally increased, in relationship with the decrease of lumen diameter. Conversely, the WCSA was not increased, indicating that there was no significant parietal growth at sites of FANs. Taken together, this favors the hypothesis that focal vasoconstriction is involved in FANs.

DISCUSSION

Here, we show that adaptive optics imaging allows qualitative and quantitative microvascular morphometry of small vessels at a near-histological scale, which allowed us to explore the structural basis of the various manifestations of hypertensive retinopathy. In a cohort of treatment-naïve individuals, our data suggests that a higher BP is accompanied by parietal thickening and lumen narrowing, and hence increased WLR of retinal vessels. This supports the notion that a diffuse vasoconstriction accompanies BP increase. As the WLR is dimensionless, any bias due to refraction is neutralized, reinforcing the robustness of our findings. This transversal study cannot, however, determine

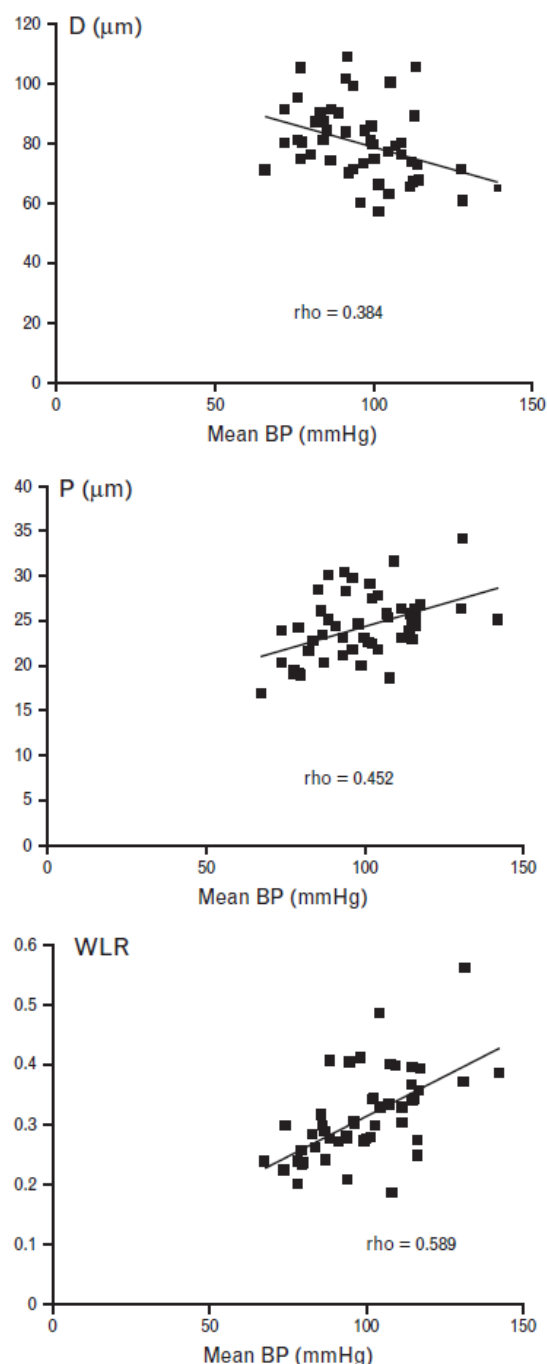


FIGURE 2 Mean blood pressure plotted against parietal (P), diameter (D) and wall-to-lumen ratio (WLR). Pearson's correlation coefficients are inserted. All regressions lines are statistically significant ($P < 0.01$).

any cause–effect relationship between BP rise and vasoconstriction.

Fundus photograph-based studies [27] reported an age-related decline of arterial lumen diameter, which may be

interpreted as indirect evidence of parietal thickening. We did observe an inverse correlation of age with WLR by univariate analysis, and multiple regression analysis confirmed the effect of age on WLR. Additional studies with larger cohorts are necessary to further document the relationship between parietal thickness and age. Nevertheless, the correlation of arterial diameters with age appears somewhat weaker than with BP.

Although it is commonly assumed that diffuse parietal thickening is initiated by a myogenic response, the pathogenesis of focal vascular changes remains uncertain. While most research and hence conceptual efforts on hypertensive microvasculopathy addressed to diffuse changes of parietal thickness, focal microvascular changes received little attention. They are indeed difficult to track by histology. Clinical studies based on fundus photographs have shown that the incidence of focal changes is correlated with age, blood pressure, and inflammation biomarkers [28]. Interestingly, it has been reported that there is a significant turnover of focal changes [29], suggesting that they are dynamic rather than degenerative processes. By adaptive optics, FANS and AVNs showed distinct anatomical features. Interestingly, neither FANS nor AVNs seemed to involve parietal growth as their primary cause. In AVNs, adaptive optics revealed a combination of loss of retinal transparency and presence of focal venous narrowings upstream and downstream of the arteriovenous crossing. Moreover, adaptive optics images of AVNs in which the arteriovenous interface could be observed showed that venous nicking could occur even in the absence of arteriovenous contact. This is in accordance with histology studies, which reports that, instead of arterial compression, changes affecting structures adjacent to the arteriovenous crossing such as axons, glial cells or the extracellular matrix may be found [30–32]. Taken together, adaptive optics and histology data argues against the prevalent model stating that the arteriole compresses the underlying vein, and instead support the hypothesis that venous nicking is mediated by retinal structures, hence implying a diffusible process. At sites of FANS, the inner and outer limits of the arteriolar wall maintained their parallelism and there was no evidence of parietal growth, suggesting that FANS were caused by focal vasoconstriction.

The morphometry of veins is gaining interest as it has been shown that venous diameter is predictive of morbidity and mortality [33,34]. The inner diameter of veins, but not the parietal thickness, can be measured with high precision by adaptive optics, which could help to determine the effect of blood pressure control on venous diameter. Adaptive optics is also of interest for the identification of focal venous narrowing at sites of AVNs, which are likely the site at which venous obstruction may occur. Hence, adaptive optics may provide insights into the factors triggering branch retinal vein occlusion, a common finding during hypertensive retinopathy.

Despite these promising results, they have to be considered as preliminary and hence a number of investigations remain to be done. We did not compare our data to ex-vivo measures by myography; nevertheless, our results on WLR measurement are very close to those observed by SDLF. This is shown in Table 3, which

TABLE 2. Univariate correlations between clinical and morphometric parameters

	D	P	WLR	WCSA
Age	−0.173	0.331*	0.348*	0.183
BMI	−0.254	0.241*	0.342*	0
SBP	−0.384**	0.438*	0.582**	0.13
DBP	−0.362*	0.437*	0.559**	0.06
Mean BP	−0.385**	0.453*	0.589**	0.05
Pulse pressure	−0.275	0.283	0.406**	0.01

D, diameter; P, parietal thickness; WCSA, wall cross-sectional area; WLR, wall-to-lumen ratio.

* $P < 0.05$.

** $P < 0.01$.

compares the demographic, clinical and morphometric characteristics of our report and of reference [16]. As SDF measures are well correlated with myographic data [17], this

suggests that the WLR measured by adaptive optics imaging is a valid surrogate of the actual WLR. A potential bias may have been the use of topical tropicamide in some eyes. To

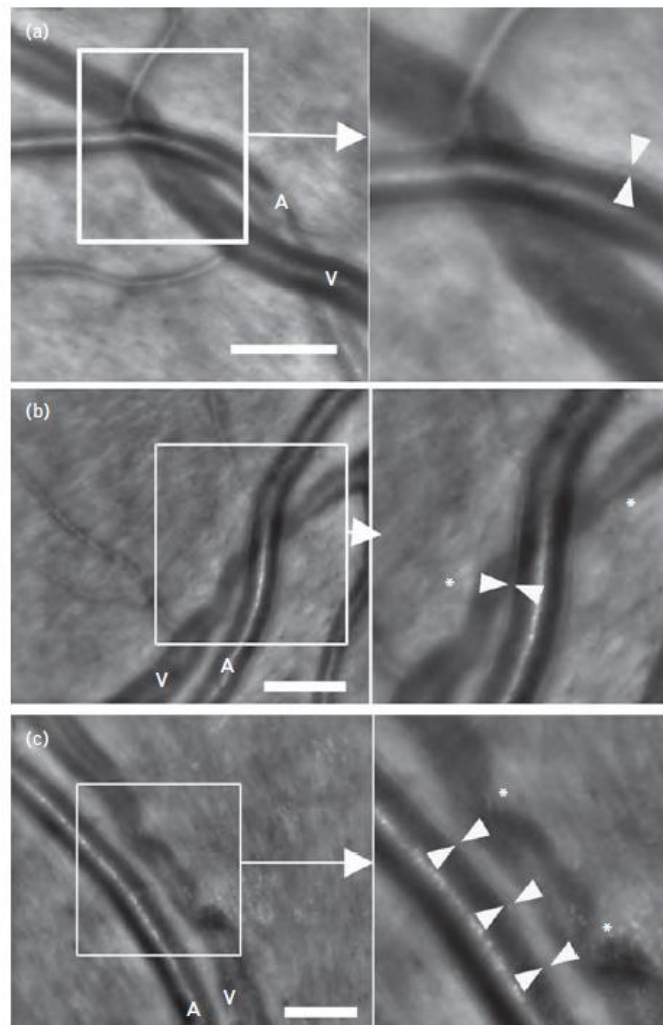


FIGURE 3 Representative adaptive optics (AO) imaging of arteriovenous crossings. Arrowheads bracket the arteriolar wall. (a) Normal arteriovenous crossing (right panel: magnification). (b) Representative cases of arteriovenous nicking (AVN). Note the focal venous narrowings (asterisks) upstream and downstream of the arteriovenous overlap. (c) Case of venous nicking occurring in the immediate vicinity of an arteriole in the absence of arteriovenous overlapping, allowing the direct observation of the arteriovenous interface; note the gap between the arteriolar wall and the vein, suggesting that there is not direct contact (bars, 125 μ m; additional cases are shown in supplementary Figure 1, <http://links.lww.com/HJH/A316>).

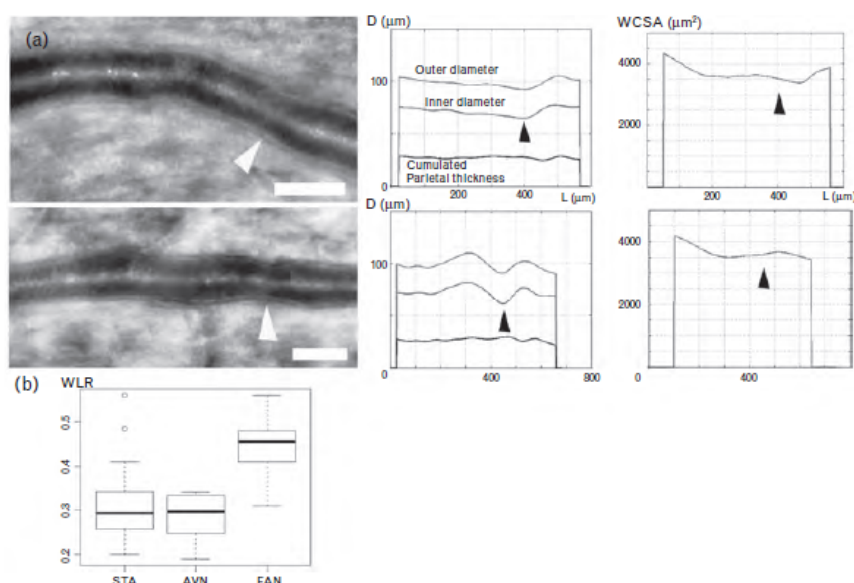


FIGURE 4 (a) Adaptive optics (AO) NIR imaging of two representative cases of focal arteriolar narrowings (FANs) with their corresponding morphograms (arrowheads in AO images and in morphograms show lumen narrowing). In both cases, the parallelism of the inner and outer vascular limits was maintained. There was no detectable increase of WCSA at the site of FAN (bar, 100 μm ; see also supplementary Figure 2, <http://links.lww.com/HJH/A316>). (b): comparison of wall-to-lumen ratio (WLR) between the superotemporal artery (STA), AVN ($n = 12$) and FANs ($n = 10$). The difference between groups is statistically significant ($P < 0.01$).

our knowledge, there is no report of the effect of topical tropicamide on major retinal vessels. In a series of nine eyes, we compared vascular morphometry before and after tropicamide administration. This showed that after tropicamide there was a mean increase of vascular diameter of 0.8%, which was not statistically significant (data not shown). We concluded that topical tropicamide had negligible effects in our measures.

A promising perspective of adaptive optics imaging is the follow-up of patients treated by antihypertensive drugs. There are indeed few reported studies of the effect of blood pressure control on retinal vascular morphometry [35,36] and on the effect of such modifications on the incidence of end-organ damage. It would be of high interest to

determine if 'microvascular responders' (i.e. patients showing arteriolar vasodilation under treatment) have a better prognosis in term of end-organ damage. One can hypothesize that if there is no change in microvascular resistances, lowering blood pressure may hamper downstream perfusion. Also, the fact that AVNs may involve damage to adjacent neuroglial structures may also be of interest to understand the pathophysiology of age-related and hypertension-related brain damage given the functional similarities of retinal and cerebral vessels [37].

In conclusion, we show here that adaptive optics imaging of retinal arterioles offers a unique opportunity to explore microvascular changes *in vivo* in humans at a near-histology level, with a simple procedure applicable

TABLE 3. Comparison of adaptive optics data from the present study and scanning laser Doppler flowmetry data from Ritt et al. [16]

	Present study		Ritt 2008	
	Normotensive	Hypertensive	Normotensive	Hypertensive
<i>n</i>	30		29	
Age	42.3 \pm 15		36.7 \pm 5.9	
Systolic pressure (mmHg)	118 \pm 13		129 \pm 6.9	
Diastolic pressure (mmHg)	74 \pm 9.5		77.8 \pm 7.6	
WLR	0.285 \pm 0.05		0.28 \pm 0.1	
Lumen diameter (μm)	83.5 \pm 11.2		85.3 \pm 11	
Wall cross-sectional area (μ^2)	3459 \pm 915		3740 \pm 1415	
<i>n</i>		19		21
Age		48 \pm 11		39.1 \pm 5.4
Systolic pressure (mmHg)		154 \pm 14		145 \pm 6.8
Diastolic pressure (mmHg)		95.5 \pm 10		87.7 \pm 8.3
WLR		0.36 \pm 0.08		0.36 \pm 0.1
Lumen diameter (μm)		74 \pm 12.6		81.8 \pm 7.8
Wall cross-sectional area (μ^2)		3338 \pm 826		4413 \pm 1725

in a routine setting. Quantitative and qualitative microvascular phenotyping by adaptive optics imaging may contribute to a better understanding of hypertensive retinopathy, and possibly improve medical management of small vessel diseases. Indeed, stratification of the risk of end-organ damage may be improved by biomarkers issued from adaptive optics imaging.

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Registered in clinicaltrials.gov (NCT01546181).

Conflicts of interest

M.P. is a consultant for the manufacturer of the camera used in the present study.

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Reviewers' Summary Evaluations

Reviewer 1

Small artery/arteriolar remodeling is a hallmark of hypertensive microangiopathy. In particular, increased wall/lumen ratio of the microvascular wall is associated with cardiovascular disease and end-organ damage. In the present study by Koch *et al.* Adaptive Optics (AO)-based fundusphotography was used to study (micro)vascular remodeling in both naive and treated hypertensive patients. The strength of this technique is that microvascular remodeling now can be studied noninvasively, which facilitates longitudinal follow-up (e.g. of treatment effects). An important requisite, however, is that this technique first needs validation to histological analysis (e.g. from gluteal biopsies).

Reviewer 2

The study proposes an innovative and promising method of evaluation of retinal small artery morphology, and provides evidence of an association between wall–lumen ratio (WLR) of retinal arterioles and blood pressure values. However, no validation of the method in respect to other available techniques is provided.

Adaptive optics should be directly compared with scanning laser Doppler flowmetry, or, even better, with the evaluation of the media-to-lumen ratio of subcutaneous small resistance arteries with micromyographic approaches, which represent, at present, the 'gold standard' and prognostically relevant approach to the evaluation of small artery morphology in human beings.

3.3 3ème article: Relationships between retinal arterioles anatomy and aortic geometry and function and peripheral resistance in hypertensives

Le but de cette étude était de démontrer une relation entre l'anatomie des artérioles rétiniennes et les résistances périphériques totales ainsi qu'avec des indices anatomiques et fonctionnels de la macro circulation en utilisant des méthodes d'imagerie non invasive de précision : l'optique adaptative pour la microcirculation rétinienne et l'IRM cardiovasculaire pour l'aorte.

Dans une population de 23 témoins et 29 hypertendus traités et contrôlés et 28 hypertendus traités et non contrôlés nous avons montré au niveau rétinien que le WLR ainsi que l'épaisseur de la paroi des artérioles étaient significativement plus élevés chez les patients hypertendus non contrôlés que chez les hypertendus contrôlés et que chez les sujets normotendus sans changement de la superficie de la section transversale de la paroi. Nous avons aussi noté une tendance non significative vers une diminution du diamètre interne chez les patients hypertendus non contrôlés.

En ce qui concerne les grosses artères, les indices de géométrie de l'aorte (diamètre de l'aorte ascendante, longueur/hauteur/largeur de l'arche aortique) indiquaient une dilatation de la section de l'aorte ascendante ainsi que d'un allongement et un élargissement de la crosse aortique chez les patients hypertendus non contrôlés. Enfin, les indices de rigidité artérielle tels que la distensibilité et la VOP de l'aorte ascendante ainsi que la VOP-cf étaient sensiblement et significativement différentes entre les 3 groupes (Table 3 Description des sujets de l'étude incluant les caractéristiques cliniques, de pression ainsi que les indices macro et microvasculaires anatomiques et fonctionnels).

	Normotensives	Controlled hypertensives	Uncontrolled hypertensives	Across groups p value
Subjects number	23	28	29	
Age, -years (SD)	45.1 (12.2)	52.9 (12.1)*	53.1(12.2)*	0,04
Antihypertensive treatment number, - n	0 (0)	1.64 (0.18)*	1.17 (0.17)*	<0.0001
Central Blood pressure				
SBP, -mmHg	112.9 (2.8)	123.1 (2.4)*	135.3 (2.3)*†	<0.0001
DBP, -mmHg	77.2 (2.1)	84.3 (1.8)*	92.6 (1.7)*†	<0.0001
MBP, -mmHg	89.3 (2.1)	97.0 (1.8)*	107.1 (1.7)*†	<0.0001
PP, - mmHg	36.1 (1.9)	39.5 (1.8)	43.4 (1.7)*	0,02
Hemodynamics				
Total peripheral resistance, - Dynes.s ⁻¹ .cm ⁻⁵	1567 (124)	1801 (108)	2047 (102)*	0,01
Cardiac Output, - ml. s ⁻¹	78.7 (9.7)	76.3 (8.8)	84.4 (8.8)	0,8
Microvasculature				
Retinal WLR	0.276 (0.012)	0.304 (0.011)	0.343 (0.010)*†	0,0002
Retinal arteriolar Internal diameter, - µm	83.7 (14.4)	78.8 (9.8)	77.2 (12.6)	0,19
Retinal arteriolar Wall Thickness, - µm	22.0 (2.32)	22.1 (3.9)	25.1 (4.3)*†	0,04
Retinal arteriolar Wall Cross Sectional Area, -µm ²	3447 (292.8)	3278 (213.8)	3477 (176.8)	0,7
Aorta Structure				
Ascending Aorta diastolic diameter, -cm	2,57 (0.06)	2,93 (0.07)*	3.09 (0.06)*	<0.0001
Aortic Arch Length, - cm	118.4 (4.9)	131.0 (4.5)	136.3 (4.5)*	<0.0001
Aortic Arch Width, -cm	64.1 (1.9)	69.3 (1.7)	77.0 (1.7) *†	<0.0001
Aortic Arch Height, -cm	39.7 (2.0)	41.8 (1.8)	44.6 (1.8)*	<0.0001
Aorta Function				
Ascending Aorta Distensibility, - kPa ⁻¹ .10 ⁻³	43.4 (3.7)	24.3 (3.3)*	18.0 (3.2)*	<0.0001
Ascending Aorta PWV, -m/s	5.37 (0.61)	7.25 (0.54)*	8.55 (0.53)*	0,001
Carotid-femoral PWV, -m/s	7.89 (0.31)	8.87 (0.27)*	9.33 (0.25)*	0,002

BMI is body mass index, SBP DBP and PP are systolic diastolic and pulse blood pressures, WLR is Wall to lumen ratio, PWV is pulse wave velocity, * p value <.05 at least vs. normotensives, † p value <.05 at least vs. controlled hypertensive

Table 3 Description des sujets de l'étude incluant les caractéristiques cliniques, de pression ainsi que les indices macro et microvasculaires anatomiques et fonctionnels

En univarié, le WLR était significativement associée au niveau tensionnel ainsi qu'avec le diamètre aortique, les indices de rigidité et les résistances périphériques totales ($r = 0,56$, $p < 0,0001$). Parmi les indices d'anatomie microvasculaire, c'est le WLR qui permettait d'établir les coefficients de corrélation les plus élevés. L'analyse multivariée a indiqué que WLR était associé aux résistances périphériques totales TPR ($p = 0,002$) indépendamment de l'âge, de l'IMC, de sexe, des traitements antihypertenseurs, de diamètre de l'aorte et de la pression systolique centrale. Pour ces analyses, des coefficients de corrélation partielle R^2 pour les variables indépendantes sont résumés dans la Figure 5 Déterminants du Wall-to-Lumen-Ratio en analyse multivariée (SBP : pression systolique centrale, TPR : résistances périphériques totales, AA : diamètre de l'aorte ascendante) indiquant que TPR a été le plus fort prédicteur de WLR. Par ailleurs, après ajustement pour l'âge et la pression il n'existait plus de relations entre le WLR et les indices de rigidité des grosses artères.

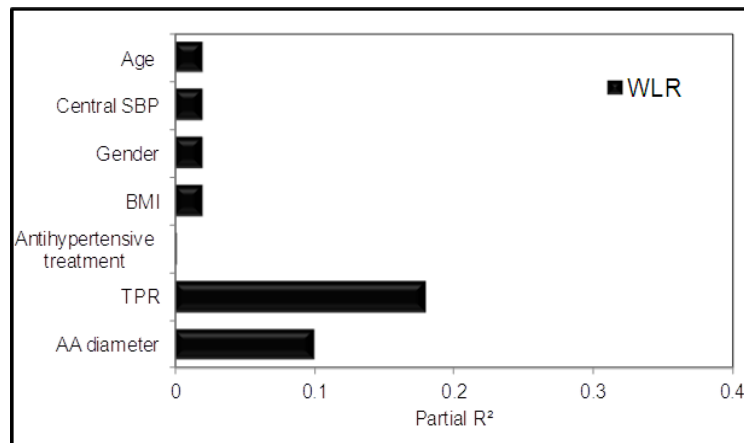


Figure 5 Déterminants du Wall-to-Lumen-Ratio en analyse multivariée (SBP : pression systolique centrale, TPR : résistances périphériques totales, AA : diamètre de l'aorte ascendante)

Conclusion :

Ce travail souligne les valeurs différentes des marqueurs micro et macrovasculaires dans l'hypertension. Si d'un côté les indices de rigidité des grosses artères centrales sont des marqueurs des effets combinés de la pression artérielle et du vieillissement, d'un autre côté, l'anatomie microvasculaire rétinienne fournit des indications sur le remodelage, les résistances périphériques totales, la charge tensionnelle et l'état de façon indépendante de l'âge et de la pression. La relation entre remodelage et TPR est un argument renforçant l'hypothèse d'une part « fonctionnelle » au remodelage microvasculaire et permet de redémontrer le lien entre hémodynamique globale et microcirculation.

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Relationships between retinal arterioles anatomy and aortic geometry and function and peripheral resistance in hypertensives

David Rosenbaum (1, 2, 5), Nadjia Kachenoura (2), Edouard Koch (3), Michel Paques(3), Philippe Cluzel (2,4,5), Alban Redheuil (2,4,5), Xavier Girerd (1)

(1) Preventive Cardiovascular Unit, Institute of Cardiometabolism and Nutrition, Groupe Hospitalier Universitaire Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France.

(2) Sorbonne Universités, UPMC Univ Paris 06, INSERM 1146, – CNRS 7371, Laboratoire d'imagerie Biomédicale, F-75013, Paris, France.

(3) Unité INSERM 968 Institut de la vision - Centre d'Investigation Clinique 503 Centre Hospitalier National des Quinze-Vingts, Assistance Publique-Hôpitaux de Paris, Paris, France.

(4) Département d'imagerie cardiovasculaire et de radiologie interventionnelle, Pôle Imagerie - Groupe Hospitalier Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France.

(5) Imaging Core Lab, Institute of Cardiometabolism and Nutrition, ICAN, Paris, France

All authors takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Corresponding author: David Rosenbaum

Unité INSERM 1146 - Laboratoire d'imagerie Biomédicale

Faculté de médecine Pierre et Marie Curie

91 Boulevard de l'Hôpital 75013 Paris

Tel. : +33 1 84 82 74 09 mail : david.rosenbaum@psl.aphp.fr

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DISCLOSURES

The authors have no conflict of interest to disclose.

Running title: retinal wall to lumen ratio and the aorta

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The funding organizations had no role in the design or conduct of this research

Abstract

Background: Microvascular remodeling and large artery stiffness are key determinants of cardiovascular hemodynamic and can now be studied with new non-invasive methods. Our objective was to study relationships between retinal arterioles anatomy, aortic geometry and function and peripheral resistance (TPR) in hypertensives.

Methods: In 80 subjects (age 52 ± 13 years; 53% males, 23 normotensives, 57 hypertensives among which 29 uncontrolled), we used: 1) the new noninvasive RTX1® adaptive optic (AO) camera (Imagine-Eyes, Orsay, France) to measure Wall-to-Lumen Ratio (WLR) on retinal microvasculature, 2) cardiovascular magnetic resonance (CMR) imaging to assess aorta stiffness, geometry, and cardiac output 3) the validated SphymoCor Xcel® device to measure Central Blood Pressure (BP) and Carotido-femoral pulse wave velocity (Cf-PWV). Total peripheral resistance (TPR) was calculated as the central mean BP/cardiac output ratio.

Results: WLR, and TPR were significantly higher and aortic distensibility lower in hypertensives. Aortic dilation and arch elongation were found in uncontrolled hypertensives. In univariate analysis, WLR correlated positively with central BP ($p < 0.001$), TPR ($p < 0.001$), Cf-PWV ($p < 0.05$), and negatively with aortic distensibility ($p = 0.003$) but not with age nor with cardiovascular risk factors. Multivariate analysis indicated that WLR was associated to TPR ($p = 0.002$) independent of age, BMI, gender, antihypertensive treatments, Aortic diameter and central SBP. As expected, age was the major correlate of AA distensibility and Cf-PWV.

Conclusions: New non-invasive vascular imaging methods are complementary to detect deleterious effects of aging or high BP on large and small arteries. AO examination could represent a useful tool for studying and follow-up of microvasculature anatomical changes.

Key words: remodeling, hypertension, optical imaging, Cardiovascular Magnetic Resonance, microcirculation

Introduction

Hypertension affects the structure and the function of both large and small arteries. The major hemodynamic determinants of blood pressure (BP) are cardiac output and total peripheral resistance (TPR) which reflect vascular tone of small arteries. The vast majority of prescribed antihypertensive treatments (such as renin-angiotensin system inhibitors or calcium channel blockers) decrease TPR via their vasorelaxation properties and some studies have shown the value of using thoracic impedance based methods that indirectly evaluate TPR to adapt antihypertensive treatments in uncontrolled hypertensive patients.¹

In hypertensive subjects, an increase of the media-to-lumen-ratio (MLR) of the small arteries has been measured in subcutaneous arteries dissected from gluteal biopsies² and more recently a non-invasive approach for small arteries imaging in-vivo has been designed and validated. Based on the Scanning Laser Doppler Flowmetry (SLDF), this technique^{3,4} was able to assess the wall-to-lumen ratio (WLR) of retinal arterioles and has confirmed associations between WLR and BP. More recently, Adaptive optics (AO), which is a novel, accurate and reproducible opto-electronic method, can provide non-invasively qualitative and quantitative microvascular morphometry of small vessels at a near-histological scale in the human retina^{5,6}.

Parallel to microvascular changes, large arteries alterations in hypertension have been studied in depth using a wide panel of methods including invasive catheterization, ultrasound⁷, applanation tonometry⁸ and more recently cardiovascular magnetic resonance (CMR) imaging. Thanks to such modalities, stiffening, hemodynamic and geometrical changes of large arteries including carotid⁹ and proximal aorta¹⁰ have been demonstrated. Recent studies indicated that a combination of central pressure measurements with CMR enable a

comprehensive evaluation of the proximal aorta throughout reliable indices of geometry¹⁰, distensibility^{11,12}, pulse wave velocity and TPR¹³. Strong associations of these CMR indices with aging and cardiovascular events have been demonstrated¹⁴.

Our aim was to investigate the relationships between WLR, large artery characteristics and total peripheral resistance in the setting of arterial hypertension. To achieve this aim, we evaluated large and small arteries characteristics in normotensive and hypertensive patients using non-invasive imaging modalities such as CMR for large arteries and AO for small arteries.

Material and Methods

Between January 2013 and February 2014, hypertensive patients and controls were prospectively recruited in a dedicated outpatient clinic (Cardiovascular Prevention Unit). This clinical study was carried out according to the principles outlined in the Declaration of Helsinki. Approval of the local Ethics Committee was obtained and informed consent was signed by all participants.

Inclusion criteria for normotensives were: no personal history of cardiovascular disease, no antihypertensive treatments, normal home blood pressure (systolic blood pressure (SBP) <135mmHg and diastolic blood pressure (DBP) <85mmHg). Inclusion criteria for hypertensives were: absence of secondary causes of hypertension and absence of personal history of cardiovascular disease, stable regimen of antihypertensive treatment for at least 6 months with a therapy containing one or two of the following classes (ARBs or Calcium Blockers). If a third antihypertensive medication was present it had to be a thiazide.

Exclusion criteria were: diabetes, antihypertensive treatment with beta-blockers, more than 4 cumulated antihypertensive treatments, contra indication to CMR exam, refusal to give consent.

Controlled hypertension was defined by having normal home blood pressure (SBP <135mmHg and DBP <85mmHg).

Study protocol

During the outpatient visit, the following data were collected: 1) basic characteristics: age, weight, height, familial and personal history of cardiovascular disease. 2)

Venous blood samples were taken in supine position to measure: urea, creatinine, potassium 3) peripheral and central blood pressure (BP) measurements. 4) Adaptative optics and CMR imaging were also performed.

Blood pressure assessment

For each subject, two different measurements of blood pressure were performed:

1) Central BP measurements were performed in a temperature controlled semi dark room, to reproduce CMR exam conditions. Measurements were performed in standard conditions after 5 minutes rest and 20 min before CMR exam using the validated SphyMoCor Xcel device (AtCor®)¹⁵.

2) Subjects were asked to record their home blood pressures according to a standardized protocol using the validated OMRON M3 brachial cuff device¹⁶ during the 3 days preceding the outpatient visit.

Overall, the following BP measurements were recorded and considered for analysis:

- Home systolic BP (SBP), home diastolic BP (DBP), home pulse pressure (PP) and home mean BP (MBP),
- Central systolic BP (cSBP), central diastolic BP (cDBP), central pulse pressure (cPP) and central mean BP (cMBP).

Small arteries: wall to lumen ratio estimation by adaptative optics

Retinal imaging was performed by an operator blinded to clinical and CMR data at the Clinical Investigation Center of the Quinze-Vingts Hospital (Paris, France) within 2 weeks before or after CMR and central pressure measurements. En face AO fundus images were obtained using a commercially available flood-illumination AO retinal camera (rtx1™; Imagine Eyes, Orsay, France).

Briefly, the rtx1 camera measures and corrects wave front aberrations with a 750 nm super luminescent diode source and an AO system operating in a closed loop. A

4°x4° fundus area (i.e. approximately 1.2mm x 1.2mm in emmetropic eyes) is illuminated at 840 nm by a temporally low coherent light emitting diode flashed flood source, and a stack of 40 fundus images is acquired in 4 seconds by a charged coupled device camera.

Among the 80 patients, 74 examinations were done without pupil dilation; in 6 patients pupil dilation was necessary and obtained with topical tropicamide monodose (2mg/0,4mL, Novartis, France). These 6 examinations were excluded from the analysis that integrated retinal AO parameters. After a 10 minutes rest, the patient was installed on the chin rest. The live video image of the pupil allowed alignment with the incident light; the live display of AO-corrected fundus image allowed adjustment of brightness, contrast and focus.

Gaze was oriented using a dedicated target in order to capture the region of interest which included a segment of the superotemporal artery of the right eye, devoid of bifurcations, with at least 250µm long and an inner diameter of at least 50µm, (see example in figure 1). The site of interest was chosen to be free of the presence of neither focal arterial nicking (FAN) nor arterio-venous crossings.

To identify the systolic pulse and delete the corresponding images, real-time videos were generated from each stack using customized imaging software. Then, the diastolic images were averaged to increase the signal to noise ratio. Such averaged diastolic AO images were semi-automatically processed using reproducible⁶ and previously described custom software¹⁷. Image processing included: image enhancement by applying a median filter followed by a nonlinear diffusion filter¹⁸ to allow smoothing the blood vessels while preserving the contrast along their edges; and a previously described image segmentation described in details in^{17 19} and based on mathematical morphology, k-means clustering and active contours models relying on parallelism information²⁰ in order to extract the internal (ID) and outer diameters (OD) of the vessel.

Finally, the ratio of total parietal thickness over the lumen diameter averaged along 250µm length defined the WLR. Wall thickness (WT) was defined as $WT = (OD - ID)/2$ and total wall cross sectional area WCSA as $WCSA = \pi * ((OD/2)^2 - (ID/2)^2)$. More details on precision of the AO device and axial correction are presented in appendix A.

Large arteries characterization

Carotid-femoral pulse wave velocity

Carotid-femoral pulse wave velocity (Cf-PWV) was measured between carotid and femoral locations using the validated SphygmoCor® Xcel device, using the distance between the two locations and the transit time between the corresponding pressure waveforms. Cf-PWV was calculated as: $PWV = 0.8 \cdot (D/t)^{21}$. All calculations, including the measurement of parameters >12 cardiac cycles, were automated. The average of two high-quality recordings was used for Cf-PWV.

Proximal aorta stiffness and geometry indices by CMR

All patients underwent CMR on a clinical 1.5T Aera Siemens magnet with a 16-channel cardiac-phased array surface coil and ECG gating. Cine SSFP acquisitions and through-plane velocity encoded data were acquired in the ascending aorta at the level of the pulmonary bifurcation perpendicular to the aortic wall. Acquisition parameters for both aortic SSFP and velocity encoding data are provided in the Appendix A.

Images were analyzed off-line by an operator blinded to clinical data. As previously described²², contours of the ascending aorta (AA) were automatically detected on SSFP images for all phases of the cardiac cycle using the validated ArtFun software (UPMC-Inserm)^{12,22,23}, providing AA area and diameter variations during the cardiac cycle. This method was also used for the automated detection of both AA and descending aorta contours from modulus images of PC sequences, which were superimposed on velocity images for AA flow analysis. The automated aortic segmentation of SSFP and PC images, combined with the Sphygmocor Xcel central BP measurements, enabled the estimation of the following parameters:

- 1) AA distensibility (AAD) = $(A_s - A_d)/(A_d \cdot cPP)$ as previously described¹¹, where A_s and A_d corresponded to SSFP systolic and diastolic AA areas (mm²) and cPP is the central pulse pressure. AA diastolic diameter was calculated from A_d .
- 2) Aortic arch width defined as the distance between the center of the ascending and descending aorta cross-sections¹⁰
- 3) Aortic arch height defined as the length of the orthogonal projection of the inflection point of the aortic arch centerline, positioned at the top of the arch, on the width of the aortic arch¹⁰
- 4) Aortic arch length was estimated from SSFP acquisitions as the distance between the ascending and proximal descending aorta locations, as previously described¹⁰.
- 5) Aortic arch pulse wave velocity (AAPWV) was estimated as the ratio between the aforementioned arch length and the transit time between ascending and descending aorta flow waveforms as previously reported¹¹. Transit time was calculated automatically using a method based on the least squares minimization approach between the systolic up-slope of the ascending and descending aorta flow curves (Art-Fun software).
- 6) Cardiac output was calculated using heart rate during the CMR velocity acquisition multiplied by the stroke volume measured as the area under the ascending aorta flow curve. Finally, total peripheral resistance (TPR) was calculated as the ratio between central mean BP and cardiac output²⁴.

Statistical analysis

All continuous variables are expressed as mean (SD), unless otherwise stated. Differences between groups were evaluated by ANOVA and the non-parametric Kruskal-Wallis test. Distribution of categorical variables between groups was evaluated using the χ^2 test. Correlations between 2 variables were assessed using a linear regression model and Pearson's correlation coefficient (r) was provided. Normality of continuous variables distribution was tested using Shapiro-Wilk's test. WLR and Stiffness parameters (Cf-PWV and AAD) were further studied for their associations with age, body mass index, central SBP, TPR and antihypertensive treatments (any treatment = 1, no treatment = 0) using multivariate regression models and partial correlations for independent determinants of WLR, AAD and Cf-PWV are provided. Prior to multivariate analyses, variance inflation due to covariates was verified by estimating a variance inflation factor <2. All statistical tests were 2-tailed and a p value <0.05 indicated statistical significance. All analyses were performed using SAS® software, JMP10.

Results

Characteristics of normotensive subjects, controlled and uncontrolled hypertensive patients enrolled in the study are detailed in Table 1 and in the appendix. While normotensive subjects were younger than hypertensive patients, BMI, sex ratio, LDL-C, HbA1c, smoking status and creatinine were similar among the 3 groups (additional table 1).

While central PP differed significantly only in uncontrolled hypertensive patients, a significant increase in all peripheral and central BP measurements was observed throughout the 3 groups with the highest BP in uncontrolled hypertensive patients, as compared to controlled hypertensives or normotensive subjects.

Large and small arteries characteristics in the 3 groups are also summarized in table 1. Regarding, small arteries, retinal WLR as well as retinal arteriolar wall thickness were significantly higher in uncontrolled hypertensive patients than in controlled hypertensives and in normotensive subjects. Whereas retinal wall cross sectional area (WCSA) did not significantly vary among the 3 groups. Moreover, a non-significant trend towards a diminution in the internal diameter was noted.

Regarding large arteries, aortic geometry indices, such as diameter and arch length, width and height indicated a dilatation of the ascending aorta cross-section as well as a lengthening and an enlargement of the aortic arch in uncontrolled hypertensive patients. Finally, indices of arterial stiffness such as CMR ascending aorta distensibility and AA-PWV as well as Cf-PWV were substantially and significantly different between the 3 groups.

Univariate associations of WLR with age, BMI, central blood pressures, large arteries indices and TPR are summarized in table 2. WLR was significantly associated with SBP, DBP and MBP ($p < .0001$) as well as with all aortic diameters and stiffness indices and TPR ($r = 0.56$, $p < .0001$). Of note, among retinal arteriolar indices WLR resulted in the highest correlation coefficients for such associations.

Multivariate analysis (Table 3) indicated that WLR was associated to TPR ($p=0.002$) independent of age, BMI, gender, antihypertensive treatments, AA diameter and central SBP. For these analyses, partial correlation coefficients R^2 for independent variables are summarized in Figure 2 indicating that TPR was the strongest predictor of WLR. TPR explained 42% of the full multivariate model variance and AA diameter 23%. For AA distensibility and Cf-PWV, age explained 64% and 59% of the full multivariate model variance, respectively (Additional figure on Appendix A). Of note, although stiffness indices were associated with WLR in univariate analysis such associations were no longer significant after adjustment for age and SBP.

Discussion

In this study, we were able to show that the WLR of small arteries of the retina was correlated to BP regimen and total peripheral resistance in hypertension. We also showed that if age and BP were the strongest associates of morphological and functional changes in large arteries, total peripheral resistance was the strongest correlate of WLR, beyond central SBP and age. Finally, among large artery parameters, we showed that local CMR evaluation of proximal aortic geometry and stiffness is relevant in hypertensive subjects. Our results are in line with previous results showing the interdependence of hypertension and arterial stiffness²¹. Here, in a population of patients with controlled and uncontrolled BP, we report a strong effect of age on stiffness but also on aortic anatomy. We extend the results from Redheuil et al^{10, 11} to hypertensive subjects showing that AAD is an efficient integrator of age and BP consequences on stiffness. In our population, Cf-PWV was strongly related to age but neither to WLR nor to TPR. Accordingly, our findings on the aorta emphasize the prominent and confounding role of age on the properties of large arteries in hypertension.

A large literature reports relationships between blood pressure and microvasculature in humansⁱ. In vitro studies by Schiffrin et al^{2,25} have consistently shown strong relationships between elevated BP and increase of the media-to-lumen-ratio (MLR) in subcutaneous arterioles. The non-invasive approach of microvasculature imaging in the retina using Scanning Laser Doppler Flowmetry (SLDF) has provided further evidence of relationships

between increased WLR and elevated BP and evidence of correlations between MLR and WLR has been shown³. Recently, Salvetti et al²⁶ also reported relationships between 24h BP, central BP and retinal WLR measured with SLDF. The strongest associations were found between daytime SBP, 24-hour SBP and WLR. Here we report that MBP, SBP and DBP are related to WLR whereas, PP is linked to AAD, by definition. PP is an indicator of the pulsatile component of the blood pressure periodic phenomenon. It is related to several hemodynamic mechanisms of which notably large arteries compliance. On the contrary, MBP is considered to represent the steady component of BP that is determined by cardiac output and vascular resistance²⁷. Another explanation for the lack of correlation between WLR and PP could be the relative youthfulness and the relative low PP in our population in comparison with other studies where associations with PP were reported²⁸.

Little data have specifically reported the coupling between small and large arteries in hypertensives. In general populations, retinal arteriolar narrowing evaluated by the arteriole to venule ratio as measured on a standard fundus photography has been linked to AAD, independently of SBP²⁹, and aortic stiffness has been associated with the central retinal arteriolar and retinal vascular fractal dimension³⁰. Salvetti et al²⁶ found that PWV was an independent determinant of WLR in a heterogeneous population of hypertensive/diabetic patients (treated and never treated). Here, in a homogenous population of hypertensives where small and large arteries were characterized at the same moment, we demonstrated a correlation between small and large arteries morphologic indices (WLR and Ascending Aorta Diameter) that was independent of age, BP and antihypertensive treatment. This observation of a morphologic continuum in different arterial beds extends the concept of interrelated changes in macro and microvasculature. Also, while stiffness indices were associated with retinal eutrophic remodeling in univariate analysis such associations were no longer significant after adjustment for age and SBP. Nevertheless, TPR was shown to be the only independent determinant of WLR, explaining 42% of the variance of the multivariate model relating WLR with age, gender, BMI, antihypertensive treatments, central SBP, AA diameter and TPR.

Conversely to all large artery parameters and consistent with previous findings^{31,30,29,26,31} but not with our previous results, WLR was not related to age in this population. The first explanation would be the lack of power and the small effective of our study. A second explanation could be that WLR is not a marker of aging but of remodeling, a dynamic phenomenon driven by pressure regimen. In essential hypertension, histological investigations of subcutaneous arteries have shown eutrophic remodeling characterized by reduced lumen diameter and increased MLR with no net increase in muscle mass³². Here, we were able to reproduce those results using an entirely noninvasive method. Arteriolar Lumen reduction can result from rearrangement of smooth muscle cells layers but it can also be secondary to vasoconstriction. Such hypotheses are in line with Rizzoni et al study which reported an association between subcutaneous MLR and the vasodilatory capacity of coronary arteries³³ a result that can also be interpreted as being due to remodeling³⁴. In the retina, an association between capillary blood flow and retinal arteries inner diameter has been reported using SLDF. In this latter study, an inverse correlation between retinal arterial vasodilatory capacity and WLR in hypertensive patients was observed³⁵. Furthermore, in our previous report on retinal small arteries in hypertensive patients thanks to the high resolution of AO, we showed that at sites of FAN, the inner and outer limits of the arteriolar wall maintained their parallelism suggesting that FANs were caused by focal vasoconstriction rather than by parietal growth⁶. Moreover, a turnover of retinal FAN³⁶ and rapid modifications of WLR after a 1 month lercanidipine treatment³⁷ has been demonstrated. Such findings may illustrate a potential interplay between structural remodeling and vascular tone in small arteries.

Although several non-invasive methods have been proposed to assess cardiac output, the direct thermodilution method remains the gold standard to assess cardiac output but requires invasive catheterization³⁸. Recently, an impedance-based device has been validated on intensive care unit patients³⁹ but the accuracy of impedance cardiography has been challenged⁴⁰. Here, we used an entirely non-invasive method¹³ to calculate resistance combining central pressure measurements with Sphygmocor Xcel device and a direct measurement of aortic flow by CMR. WLR is a static parameter whose components are lumen diameter and wall thickness. From our experience on a largest population, lumen varies after few days and wall thickness after few weeks in case of treatment inducing blood pressure drop. Our results suggest that arterioles <80µm assessed with the adaptive optics camera are resistive vascular territories that potentially determine TPR. However, when considering the physiological link between TPR and vascular anatomy, lumen should be the main determinant of TPR. Here, TPR was found to be correlated with WLR but not with lumen. This result may be explained by the fact that 1) diameter of arterioles assessed with AO would not be directly responsible for TPR and 2) it has been admitted from previous studies on subcutaneous biopsies and confirmed by our personal data that WLR is constant along the vascular tree in a microcirculatory territory.

Increased WLR in the retina, carotid IMT and aortic stiffness predict target organ damage and cardiovascular events in patients with hypertension⁴¹ but so far, none has been evaluated as a therapeutic tool in antihypertensive treatment management. However, impedance measurements have been already successfully used for personalized management of resistant hypertension⁴². Furthermore, while a total normalization of small arteries alterations was found in hypertensive patients treated by Angiotensin Converting Enzyme Inhibitors or Calcium Blockers, normalization was incomplete or absent in patients treated with beta blockers or diuretics⁴³. In this context, adaptive optics WLR, thanks to its reproducibility and its non-invasive nature as well as its strong association with resistance could be of major usefulness in the follow-up of arteriolar tree tonus and morphological modifications under antihypertensive treatments.

Considering cardiovascular risk assessment, current markers and risk equations are poor predictors of cerebrovascular events whereas Ota et al showed that hypertensives retinopathy features predicted cerebrovascular events⁴⁴. Evidence is also increasing about structural retinal microvasculature changes being associated with cardiovascular risk even early in life⁴⁵ as well as with emerging subclinical MRI brain infarcts and white matter lesions⁴⁶. In line with such findings, WLR could represent a potential predictor of cardiovascular events in hypertensives.

Our study has several limitations. First of all, because of the cross-sectional design of our study we can only describe associations and no conclusion on causality can be drawn. Second, we included a relatively small number of patients. This is certainly the main explanation for the lack of relation between WLR and age. However, this enabled the construction of homogenous and comparable groups that were well characterized while avoiding potential biases such as diabetes, smoking status or gender imbalance. Only age was slightly different between groups but this was accounted for in multivariate analysis by age adjustment. The third limitation is on BP levels assessment and timing. Central BP was recorded at the CMR time and not during the AO examination but both were very close in time and performed under the same conditions. Neither night time BP nor 24h-central BP were recorded however home daytime-BP used in our study has been previously shown to be the strongest predictor of WLR²⁶. Another limitation consists in the exclusion of regions with FAN in AO images analysis. We previously demonstrated that WLR in FAN regions was

significantly increased ⁶. Still, despite the analysis in FAN free areas, we were able to show a significant increase in WLR in uncontrolled hypertensive subjects.

Although it is well known that endothelial dysfunction is an initial step of the process of athero- and arteriosclerosis in hypertensives, the evaluation of endothelial function was unfortunately not performed in our study. Blood samples for Hs-CRP were taken and whereas Hs-CRP was slightly higher in the hypertensive group, no independent associations with WLR were found in our study group (data not shown). Also, we studied patients under antihypertensive treatment. All patients were treated by similar vasodilators on a stable regimen. It may have blurred some correlations, especially with resistance and have hampered the identification of medication effects on WLR. Moreover, due to the small size of our population we were not able to address potential effects of specific antihypertensive drug class. Retinal microvasculature WLR is known to be elevated in diabetes because of hypertrophic remodeling. Accordingly, it would have been interesting to compare such population with hypertensives. However, type 2 diabetic patients often display other risk factors, which may have hampered comparisons in small population.

Our results highlight the differential meaning between macro- and micro-vascular indices. While cf-PWV and AAD are markers of arterial aging in proximal large arteries, retinal arterioles WLR of the retinal arterioles is a marker of blood pressure regimen, total resistance and remodeling of the distal arterial tree. Consequently, these different markers may be useful to address different therapeutic targets. Quantitative, qualitative, in vivo and non-invasive characterization by Adaptive Optics (AO) and CMR imaging contribute to a better understanding of hypertension consequences on large and small arteries. Adaptive Optics examination is a quick, reproducible and noninvasive technique that could represent a useful tool to follow microvasculature short and long term changes in relation with antihypertensive treatments and beyond WLR measurements, AO will allow examination of several arteriolar characteristics such as FAN, arterio-venous nicking, wall thickness regularity, or bifurcation conformation. Future studies should be performed to determine the relative usefulness of micro and macro arterial markers with respect to patient outcome and management strategies.

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TABLES

	Normotensives	Controlled hypertensives	Uncontrolled hypertensives	Across groups p value
Subjects number	23	28	29	
Age. -years (SD)	45.1 (12.2)	52.9 (12.1)*	53.1 (12.2)*	0.04
Antihypertensive treatment number. - n	0 (0)	1.64 (0.18)*	1.17 (0.17)*	<0.0001
Central Blood pressure				
SBP.-mmHg	112.9 (2.8)	123.1 (2.4)*	135.3 (2.3)*†	<0.0001
DBP.-mmHg	77.2 (2.1)	84.3 (1.8)*	92.6 (1.7)*†	<0.0001
MBP.-mmHg	89.3 (2.1)	97.0 (1.8)*	107.1 (1.7)*†	<0.0001
PP.- mmHg	36.1 (1.9)	39.5 (1.8)	43.4 (1.7)*	0.02
Hemodynamics				
Total peripheral resistance.- Dynes.s ⁻¹ .cm ⁻⁵	1567 (124)	1801 (108)	2047 (102)*	0.01
Cardiac Output.- ml. s ⁻¹	78.7 (9.7)	76.3 (8.8)	84.4 (8.8)	0.8
Microvasculature				
Retinal WLR	0.276 (0.012)	0.304 (0.011)	0.343 (0.010)*†	0.0002
Retinal arteriolar Internal diameter.- μm	83.7 (14.4)	78.8 (9.8)	77.2 (12.6)	0.19
Retinal arteriolar Wall Thickness.- μm	22.0 (2.32)	22.1 (3.9)	25.1 (4.3)*†	0.04
Retinal arteriolar Wall Cross Sectional Area. -μm ²	3447 (292.8)	3278 (213.8)	3477 (176.8)	0.7
Aorta Structure				
Ascending Aorta diastolic diameter. -cm	2.57 (0.06)	2.93 (0.07)*	3.09 (0.06)*	<0.0001
Aortic Arch Length. - cm	118.4 (4.9)	131.0 (4.5)	136.3 (4.5)*	<0.0001
Aortic Arch Width. -cm	64.1 (1.9)	69.3 (1.7)	77.0 (1.7) *†	<0.0001
Aortic Arch Height. -cm	39.7 (2.0)	41.8 (1.8)	44.6 (1.8)*	<0.0001
Aorta Function				
Ascending Aorta Distensibility. - kPa ⁻¹ .10 ⁻³	43.4 (3.7)	24.3 (3.3)*	18.0 (3.2)*	<0.0001
Ascending Aorta PWV. -m/s	5.37 (0.61)	7.25 (0.54)*	8.55 (0.53)*	0.001
Carotid-femoral PWV. -m/s	7.89 (0.31)	8.87 (0.27)*	9.33 (0.25)*	0.002

BMI is body mass index, SBP DBP and PP are systolic diastolic and pulse blood pressures, WLR is Wall to lumen ratio, PWV is pulse wave velocity , * p value <.05 at least vs. normotensives , † p value <.05 at least vs. controlled hypertensive

Table 1. Subjects description including basic characteristics, blood pressure measurements, anatomical and functional micro- and macro-vascular indices

	WLR
Demographic characteristics	
Age. -years	0.14
BMI.- kg/m ²	0.22
Blood pressure	
Central SBP.-mmHg	0.48 †
Central DBP.-mmHg	0.50 ‡
Central. MBP.-mmHg	0.51 ‡
Central PP.- mmHg	0.22
Carotid structure	
Common Carotid Mean IMT. -mm	0.05
Aorta Structure	
Aortic Arch Length. - cm	0.17
Aortic Arch Width. -cm	0.22
Aortic Arch Height. -cm	0.06
Ascending Aorta diastolic diameter. -cm	0.39†
Aorta Function	
Ascending Aorta Distensibility. - kPa ⁻¹ .10 ⁻³	- 0.39 †
Ascending Aorta PWV. -m/s	0.33 †
Carotid-femoral PWV. -m/s	0.30 *
Peripheral resistance	
Total peripheral resistance.- Dynes.s ⁻¹ .cm ⁻⁵	0.56 ‡

WLR is Wall to Lumen Ratio,SBP DBP MBP and PP are systolic diastolic mean and pulse blood pressures
* p value <.05 at least, † p value <.01 at least , ‡ p value <.001 at least

Table 2. Correlation coefficients resulting from univariate associations between retinal and wall-to-lumen ratio (WLR) as well as wall cross sectional area (WCSA) , and age, BMI, central blood pressures, large arteries indices and total peripheral resistance.

	Wall to Lumen Ratio		
	R ²	β (SD)	p
Overall Model	0.43		0.0001
Age.- years		- 0.0005 (0.0006)	0.36
Gender (male=1)		- 0.0112 (0.0128)	0.38
BMI. - kg/m ²		0.0013 (0.0013)	0.34
cSBP. - mmHg		0.0005 (0.0005)	0.33
Antihypertensive treatment (for yes=1)		- 0.0033 (0.0158)	0.83
Total peripheral resistance. - Dynes.s ⁻¹ .cm ⁻⁵		5.4.10 ⁻⁵ (1.6 .10 ⁻⁵)	0.002
AA Diameter.- cm		0.05 (0.22)	0.02

BMI is body mass index, cSBP is central systolic blood pressure, AA is ascending aorta

Table 3. Correlates of wall to lumen ratio

FIGURES

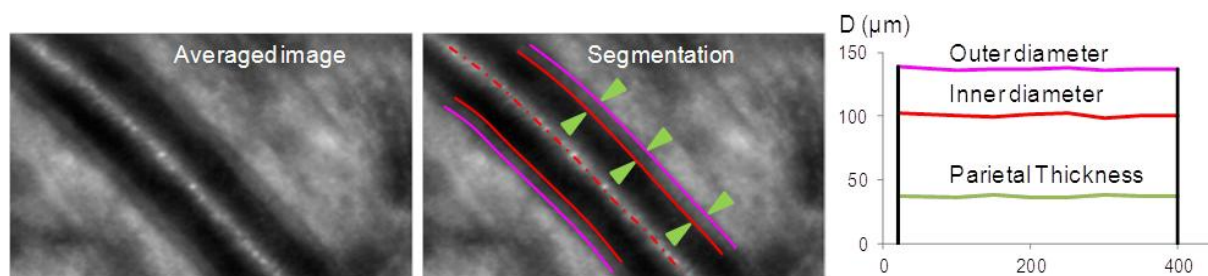


Figure 1. AO imaging and segmentation of a retinal arteriole. Left: averaged image after acquisition. Middle: image segmentation. Note that parietal structures (between arrowheads) can be seen. C, morphogram of the segmented vessel (D: diameter; L: length).

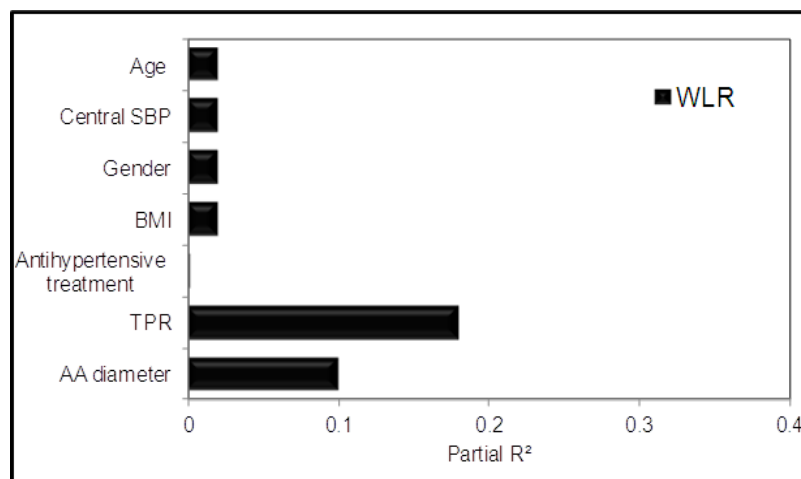


Figure 2. Determinants of WLR. Partial correlation coefficients R² are provided for each variable along with its statistical significance, which was indicated by * p value <.05, † p value <.01, ‡ p value <.001. AA is ascending aorta, BMI is Body Mass index, TPR is total peripheral resistance, cSBP is central systolic blood pressure.

3.4 4^{ème} article: Effects of age, blood pressure and antihypertensive treatments on retinal arterioles remodeling assessed by adaptive optics.

Le but de ce travail était de déterminer l'influence de l'âge et de la pression artérielle sur les différents indices anatomiques de la paroi artériolaire rétinienne en utilisant une nouvelle technique d'imagerie du fond d'œil : l'optique adaptative dans une large population de patients avec des facteurs de risque (1000 patients).

Notre population était composée de 61% d'hypertendus, de 45% de dyslipidémiques et de 15% de diabétiques. L'épaisseur, le diamètre et le WLR moyen des artérioles rétinienne étaient de $23.2 \pm 3.9\mu\text{m}$, $78.0 \pm 10.9\mu\text{m}$ et 0.300 ± 0.054 respectivement.

L'âge était positivement et indépendamment corrélé à l'épaisseur et au WLR mais n'exerçait pas d'influence sur la lumière. La pression quant à elle était positivement et indépendamment corrélée au WLR et à l'épaisseur mais négativement corrélée à la lumière. L'analyse multivariée a révélé une interaction entre âge et pression montrant que l'effet de la pression sur la lumière diminuait avec l'âge (

Figure 6).

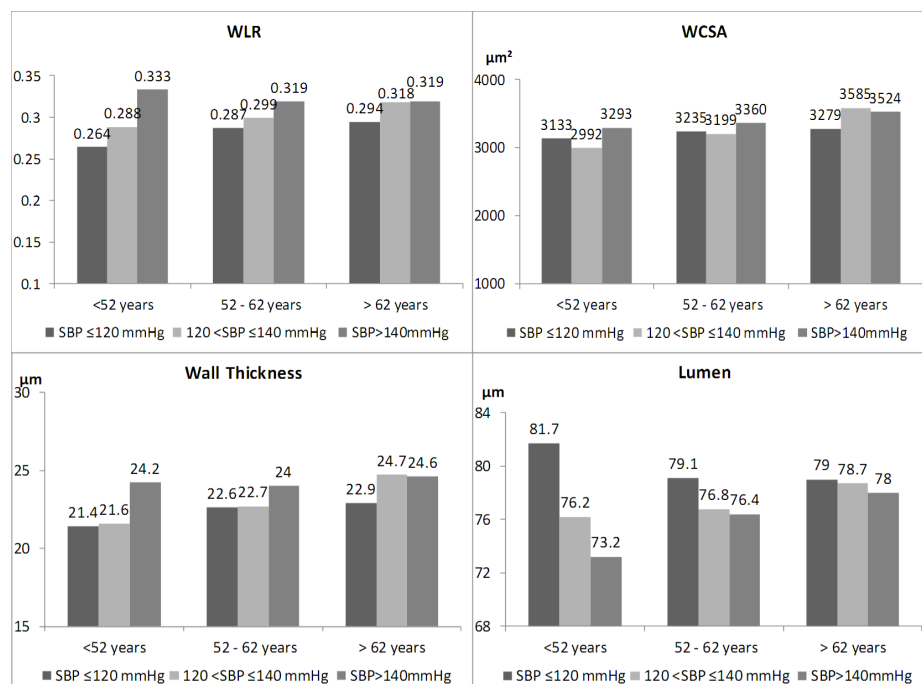


Figure 6 Epaisseur (WT), lumière (Lumen), surface transscetionnelle (WCSA) et rapport mur/lumière (WLR) artériolaire rétinien en fonction des tertiles d'âges et de pression.

L'ANOVA à 2 composantes était significative pour tous les paramètres ($p < 0.0001$). L'effet de la pression artérielle et l'interaction entre l'âge et la pression étaient significatifs pour tous les paramètres. L'effet de l'âge était significatif pour tous les paramètres à l'exception de la lumière.

En ce qui concerne les autres facteurs de risque, nous avons retrouvé chez les patients diabétiques une augmentation du WLR avec une augmentation importante de l'épaisseur pariétale et de la surface transsectionnelle signant un remodelage hypertrophique. Les femmes avaient un WLR strictement identique à celui des hommes alors que leur diamètre et leur épaisseur étaient plus importants (Table 4 Indices anatomiques microvasculaires rétiens et pression artérielle en fonction du sexe et de la présence d'un diabète, d'une dyslipidémie ou d'une obésité

	N	Retinal Arteriolar Microvasculature				Blood Pressure.-mmHg	
		WLR	ID.- μm	WT.- μm	WCSA.- μm^2	Systolic	Diastolic
Diabetes							
Yes	186	0.307 \pm .055 *	79.7 \pm 11.2 *	24.2 \pm 3.9 ‡	3530 \pm 911 ‡	134.1 \pm 19.0 †	71.2 \pm 13.3
No	814	0.290 \pm .050	77.6 \pm 10.9	22.9 \pm 3.9	3239 \pm 845	129.5 \pm 20.5	72.0 \pm 13.7
Dyslipidemia							
Yes	456	0.300 \pm .053	78.5 \pm 11.2	23.3 \pm 4.2	3337 \pm 917	130.5 \pm 20.8	69.8 \pm 12.2 ‡
No	544	0.301 \pm .054	77.6 \pm 10.8	23.1 \pm 3.7	3261 \pm 817	130.2 \pm 19.8	74.2 \pm 14.5
Obesity							
Yes	265	0.302 \pm .054	77.0 \pm 11.4	23.1 \pm 3.9	3249 \pm 880	136.4 \pm 20.2 ‡	76.7 \pm 13.0 ‡
No	785	0.303 \pm .051	78.0 \pm 10.67	23.2 \pm 3.9	3310 \pm 856	129.0 \pm 20.2	70.5 \pm 13.6
Gender							
Female	486	0.300 \pm .049	79.1 \pm 10.8 ‡	23.5 \pm 3.6 ‡	3383 \pm 824 ‡	128.0 \pm 20.8 ‡	72.0 \pm 13.6
Male	514	0.300 \pm .057	77.0 \pm 11.1	22.8 \pm 4.2	3208 \pm 890	132.4 \pm 19.8	71.6 \pm 13.6

WLR is Wall-to-Lumen Ratio, ID is Internal Diameter, WT is Wall Thickness, WCSA is Wall Cross Sectional Area, * p < .05, † p < .01, ‡ p < .001 between groups

Table 4 Indices anatomiques microvasculaires rétiens et pression artérielle en fonction du sexe et de la présence d'un diabète, d'une dyslipidémie ou d'une obésité

A court terme, une baisse de pression de 29.3 ± 17.3 mmHg pour la pression 14.4 ± 10.0 mmHg pour la diastolique s'accompagnait d'une diminution de $6.0 \pm 8.0\%$ du WLR en raison d'une augmentation de la lumière de $+ 4.4 \pm 5.9 \%$ sans modifications de l'épaisseur artérielle (

Figure 7 Variations relatives en % des pressions systoliques et diastoliques (SBP et DBP) ainsi que des indices anatomiques (WLR : rapport mur/lumière, WT : épaisseur pariétale, ID : lumière et WCSA : surface transsectionnelle) dans les 2 groupes de patients)

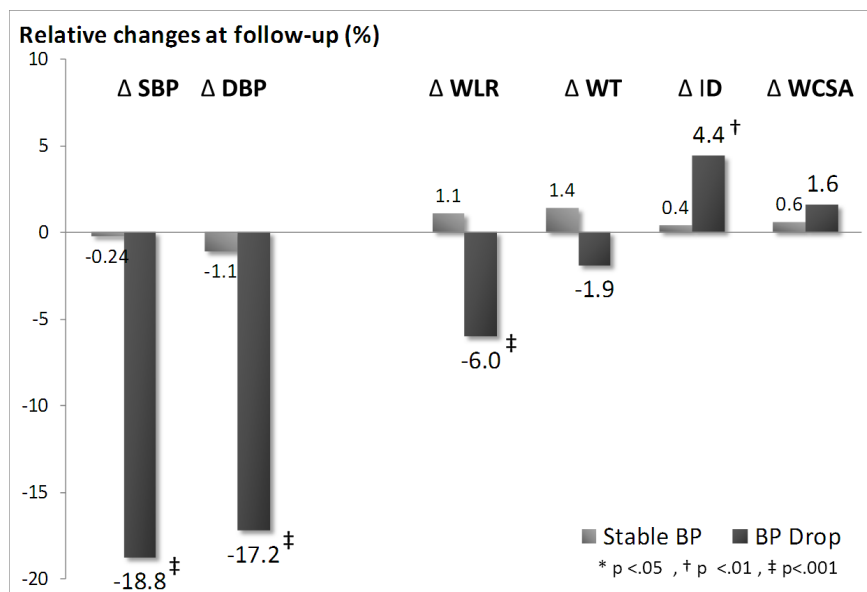


Figure 7 Variations relatives en % des pressions systoliques et diastoliques (SBP et DBP) ainsi que des indices anatomiques (WLR : rapport mur/lumière, WT : épaisseur pariétale, ID : lumière et WCSA : surface transsectionnelle) dans les 2 groupes de patients

En ce qui concerne les traitements antihypertenseurs, aucun des indices anatomiques rétiens n'étaient différents de ceux observés chez des normotendus non traités chez les hypertendus traités et contrôlés par une monothérapie, Il n'y avait pas de différences anatomique non plus entre les patients qu'ils soient traités en monothérapie par un inhibiteur du système rénine angiotensine ou non. L'analyse multivariée () a permis de confirmer les rôles respectifs de l'âge et de la pression et aussi de montrer l'absence d'influence des traitements antihypertenseurs sur les paramètres rétiens.

	Model R ²	SBP (β ± SD)	Age (β ± SD)	Covariates
WLR	0.19 ‡	0.0009 ± 0.00009 ‡	0.0007 ± 0.0001 ‡	gender, Diabetes, (SBPxAge) †, BMI, Treatment
Wall.- μm	0.16 ‡	0.044 ± 0.007 ‡	0.072 ± 0.011 ‡	gender ‡, Diabetes †, (SBPxAge), BMI, Treatment
Lumen.- μm	0.06 ‡	-0.078 ± 0.020 ‡	0.059 ± 0.031	gender, Diabetes *, (SBPxAge) ‡, BMI, Treatment
WCSA.- μm ²	0.09 ‡	4.0 ± 1.5 ‡	13.9 ± 2.44 ‡	gender ‡, Diabetes †, (SBPxAge), BMI, Treatment

WLR is Wall to lumen Ratio, WCSA is wall cross sectional area, SBP is Systolic Blood Pressure, * p < .01, † p < .001 ‡ p < .0001

Table 5 Analyse multivariée des déterminants des indices anatomiques rétiens incluant l'âge, la pression, le sexe, l'IMC, la présence d'un diabète et le fait de recevoir un traitement antihypertenseur

Conclusion :

Bien que l'hypertension artérielle et l'âge aient des effets similaires sur l'indice de remodelage artériolaire global, le WLR ceci reflète en fait les effets combinés mais distincts de l'âge (augmentant l'épaisseur pariétale) et de la pression (épaississement et une diminution de lumière). Ces constatations, combinées à l'observation d'une modification rapide du remodelage et du diamètre artériolaire en cas de chute de pression incite à penser que le remodelage rétinien est le fruit de processus fonctionnels rapides et structurels de long terme.

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EFFECTS OF AGE, BLOOD PRESSURE AND ANTIHYPERTENSIVE TREATMENTS ON RETINAL ARTERIOLES REMODELING ASSESSED BY ADAPTIVE OPTICS

David ROSENBAUM (1, 2, 3), Alessandro MATTINA (1,2), Edouard KOCH(4), Florence ROSSANT (5) Antonio GALLO (1,2), Nadjia KACHENOURA (2,3), Michel PAQUES (4), Alban REDHEUIL (2, 3,6), Xavier GIRERD (1)

(1) Preventive Cardiovascular Unit, Institute of Cardiometabolism and Nutrition, Groupe Hospitalier Universitaire Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France.

(2) Sorbonne Universités, UPMC Univ Paris 06, INSERM 1146, – CNRS 7371, Laboratoire d'imagerie Biomédicale, F-75013, Paris, France.

(3) Imaging Core Lab, Institute of Cardiometabolism and Nutrition, ICAN, Paris, France

(4) Unité INSERM 968 Institut de la vision - Centre d'Investigation Clinique 503 Centre Hospitalier National des Quinze-Vingts, Assistance Publique-Hôpitaux de Paris, Paris, France.

(5) ISEP, Paris

(6) Département d'imagerie cardiovasculaire et de radiologie interventionnelle, Pôle Imagerie - Groupe Hospitalier Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France.

Short title: Age, pressure and arteriolar remodeling

Corresponding author: David Rosenbaum

Unité INSERM 1146 - Laboratoire d'imagerie Biomédicale

Faculté de médecine Pierre et Marie Curie

91 Boulevard de l'Hôpital 75013 Paris

Tel. : +33 1 42 17 57 74 Fax + 33 1 42 17 72 34 mail : david.rosenbaum@aphp.fr

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Abstract

In humans, adaptive optics camera enables precise large-scale non-invasive retinal microcirculation evaluation to assess ageing, blood pressure (BP), and antihypertensive treatments respective roles on retinal arterioles anatomy.

We used adaptive optics camera rtx1™ (Imagine-Eyes, Orsay, France) to measure wall thickness, internal diameter and to calculate Wall-to-Lumen Ratio (WLR) and Wall Cross Sectional Area (WCSA) of retinal arterioles. This assessment was repeated within a short period in two subgroups of hypertensive subjects without or with a drug-induced BP drop.

In 1000 subjects, mean wall thickness, lumen diameter and WLR were $23.2 \pm 3.9 \mu\text{m}$, $78.0 \pm 10.9 \mu\text{m}$ and 0.300 ± 0.054 respectively. BP and age both independently increased WLR by thickening arterial wall. In opposite, hypertension narrowed lumen in younger as compared to older subjects (73.2 ± 9.0 vs. $81.7 \pm 10.2 \mu\text{m}$; $p < 0.001$) while age exerted no influence on lumen diameter. Short-term BP drop ($-29.3 \pm 17.3 / -14.4 \pm 10.0 \text{ mmHg}$) induced a WLR decrease ($-6.0 \pm 8.0 \%$) due to lumen dilatation ($+4.4 \pm 5.9 \%$) without wall thickness changes. By contrast no modifications were observed in subjects with stable BP. In treated and controlled hypertensives under monotherapy WLR normalization was observed due to combined wall decrease and lumen dilatation independently of antihypertensive pharmacological classes. In multivariate analysis, hypertension drug regimen was not an independent predictor of any retinal anatomical indices.

Retinal arteriolar remodeling comprised BP and age-driven wall thickening as well as BP-triggered lumen narrowing in younger subjects. Remodeling reversal observed in controlled hypertensives seems to include short-term functional and long-term structural changes.

Key words: adaptive optics camera, ageing, arteriolar remodeling, microcirculation,

Introduction

Effects of ageing and hypertension have been widely studied in large arteries [1] but because of the invasive nature of the most of the methods used to assess it much fewer data exists on microcirculation in humans. In subcutaneous arteries dissected from gluteal biopsies in hypertensive subjects [2], an increase of small arteries media-to-lumen-ratio (MLR) has been measured in-vitro and has been shown to be predictive of end-organ damage [3]. The prevalent physiopathological concept of such parietal thickening postulates that a rise in blood pressure stimulates myogenic vasoconstriction, which tends to normalize parietal stress [4], without significant modification of the parietal components leading to inward eutrophic remodeling.

More recently, a non-invasive approach to the microcirculation has been developed through retinal arterioles imaging and has been widely used, especially in hypertension [5] and in few selected small populations to assess the effect of aging [6]. Recently developed Adaptive Optics Camera (AOC), which is a novel, accurate opto-electronic method, can provide non-invasively qualitative and quantitative microvascular morphometry of the different anatomical component of small vessels at a near-histological scale in the human retina [7]. Few studies of the effect of blood pressure (BP) decrease on retinal vascular morphometry reported long-term improvement in arteriolar narrowing in small hypertensive or diabetic populations and data on short-term modifications are scarce [6–11]. AOC noninvasive technology enables large scale in vivo measurements of retinal small arteries remodeling through the evaluation of wall-to-lumen-ratio (WLR) and its components: wall thickness (WT) and lumen diameter (ID). Moreover, AOC was shown to be highly reproducible [6] [12] ensuring accurate and reliable follow-up measurements.

Our aims were: to use AOC: 1) to specifically investigate the relative effects of age, BP and other risk factors on retinal arterioles anatomical remodeling (WLR) and its components (WT and ID) in a large population, 2) to investigate the effect of antihypertensive treatments in a large cohort of subjects treated with antihypertensive drugs and to prospectively assess changes on retinal arterioles anatomy after a short term BP drop using consecutive AO measurements in a subgroup of hypertensive subjects.

Material and Methods

Between June 2014 and April 2015, 1000 patients were consecutively recruited in our outpatient clinic (Cardiovascular Prevention Unit, Institute of Cardiometabolism and

Nutrition, Groupe Hospitalier Universitaire Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France). This clinical study was carried out according to the principles outlined in the Declaration of Helsinki. Approval of the local Ethics Committee was obtained and informed consent was signed by all participants.

Exclusion criteria were refusal to give consent and impossibility to undergo AOC assessment.

Study protocol

During the outpatient visit, the following data were collected:

Baseline adaptative optics fundus examination.

Basic characteristics: age, weight, height, familial and personal history of cardiovascular disease.

Venous blood samples were taken in supine position to measure: creatinine, potassium, total cholesterol HDL-C, triglycerides, as well as fasting glucose and HBA1c. LDL-C was calculated using the Friedwald equation or dosed if triglycerides were above 4.56mmol/L.

Peripheral BP measurements as described below.

In a subgroup of 75 hypertensive patients, a second AOC examination was performed before 90 days after first inclusion, along with peripheral BP measurements. Second AOC images were acquired on the exact same conditions as the first examination and at the very same place on the arteriolar tree as described below. Image analysis was performed by the same trained operator blinded to clinical data. Image quality and location of the WLR measures on the arteriolar tree were checked by a second operator blinded to clinical data and to the first AO measurements. For analysis, patients were divided into 2 groups:

- A group with stable BP: hypertensives with stable BP who had no changes in their drug regimen and in whom absolute office mean blood pressure (MBP) change was < 10 mmHg at follow up.
- A group with a drop in BP: subjects with uncontrolled office BP at baseline in whom a decrease of the office MBP ≥ 10 mmHg was observed due to a change in antihypertensive drug regimen between the 2 visits.

Blood pressure measurements

For all subjects, office brachial BP were assessed in standard conditions in seated position during the outpatient visit using an oscillometric device simultaneously to AOC assessment. Hypertension was defined as the presence of Systolic BP (SBP) >140mmHg and/or Diastolic BP (DBP) > 90mmHg during the AOC examination or by the intake of antihypertensive treatment. Controlled hypertension was defined as having SBP< 140mmHg and DBP <90mmHg. Antihypertensive treatments were recorded in all subjects and divided into the following classes: Renin Angiotensin System (RAS) blockers, Calcium Channel Blockers, Diuretics, Betablockers, other class. Patient file were used to select patient who were under a stable monotherapy for more than 3 months.

Small arteries: wall to lumen ratio estimation by adaptive optics

Retinal imaging was performed by an operator blinded to clinical data at the Cardiovascular Prevention Unit. En face AOC fundus images were obtained using a commercially available flood-illumination AOC retinal camera (rtx1™; Imagine Eyes, Orsay, France).

Briefly, the rtx1™; camera measures and corrects wave front aberrations with a 750 nm super luminescent diode source and an AOC system operating in a closed loop. A

4°x4° fundus area (i.e. approximately 1.2mm x 1.2mm in emmetropic eyes) is illuminated at 840 nm by a temporally low coherent light emitting diode flashed flood source, and a stack of 40 fundus images is acquired in 4 seconds by a charged coupled device camera. No pupil dilation was necessary. After a 5 minutes rest, the patient was installed on the chin rest. The

live video image of the pupil allowed alignment with the incident light; the live display of AOC-corrected fundus image allowed adjustment of brightness, contrast and focus.

Gaze was oriented using a dedicated target in order to capture the region of interest which included a segment of the superotemporal artery of the right eye, devoid of bifurcations, with at least 250µm long and an inner diameter of at least 50µm, (see example in figure 1). The site of interest was chosen to be free of the presence of neither focal arterial nicking (FAN) nor arterio-venous crossings.

To identify the systolic pulse and delete the corresponding images, real-time videos were generated from each stack using customized imaging software. Then, the diastolic images were averaged to increase the signal to noise ratio and were analyzed. Briefly, image processing included: (i) image enhancement by applying a median filter followed by a nonlinear diffusion filter [13] to allow smoothing the blood vessels while preserving the contrast along their edges; (ii) image segmentation based on mathematical morphology, k-means clustering, and active contours models relying on parallelism information, in order to extract the lumen (ID) and outer diameters (OD) of the vessel. Finally, Wall thickness (WT) was defined as $WT = (OD - ID)/2$, total wall cross sectional area WCSA as $WCSA = \pi * ((OD/2)^2 - (ID/2)^2)$ and the ratio of total parietal thickness over the lumen diameter averaged along 250µm length defined the WLR. Intra observer reproducibility of microvasculature anatomical indices, although already published[14] was reassessed in a subgroup of 30 patients with treated and controlled hypertension (additional table 1).

Clinical characterization

Patient clinical records were used to assess presence of diabetes and dyslipidemia. Diabetes was defined as being treated by antidiabetic drugs. Dyslipidemia was defined by being treated by any lipid lowering drugs. Current smoking was defined as any cigarette in the past month. Obesity was defined as Body Mass Index above 30kg.m⁻²

Statistical analysis

All continuous variables are expressed as mean (SD), unless otherwise stated.

Differences between groups were evaluated by ANOVA and Student test. Distribution of categorical variables between groups was evaluated using the χ^2 test. Normality of continuous variables distribution was tested using Shapiro-Wilk's test. Correlations between 2 variables were assessed using a linear regression model and Pearson's correlation coefficient (r) was provided. 2 ways ANOVA was used to study WLR, WT, ID and WCSA according to age and SBP tertiles. Finally WLR, WT, ID and WCSA were further studied for their associations with age, SBP, gender (male =1), BMI, presence of diabetes (yes=1), any hypertensive treatment (yes=1) and interaction between age and BP using multivariate regression model. Differences in retinal anatomical indices and BP between baseline and follow-up visit were evaluated using a matched pair Student test analysis. All statistical tests were 2-tailed and a p value <0.01 indicated statistical significance. All analyses were performed using SAS® software, JMP10.

Results

Population characteristics are displayed in table 1. In 1000 subjects, mean WT, ID and WLR were 23.2±3.9 µm, 78.0±10.9 µm and 0.300±0.054 respectively.

Effects of age on retinal arteriolar remodeling

Ageing was accompanied by an increase in WLR as displayed in figure 1. This augmentation was driven by a wall thickening that wasn't accompanied by a lumen decrease. Moreover, WCSA increased also with age. Multivariate analysis (table 2) showed positive and

independent correlations between age and WLR, WT and WCSA whereas no associations were found with lumen (see additional table 2 for univariate analysis).

Effects of Blood pressure on retinal arteriolar remodeling

BP elevation correlated with the increase in WLR (figure 1), independently of ageing. In opposite to ageing, BP-driven WLR increase was caused by concomitant WT increase and ID diminution. Univariate and multivariate analysis (table 2 and additional table 2) showed positive and independent relationships between BP and WLR, WT and WCSA as well as negative correlations with ID. Moreover, interaction between age and BP contributed significantly to ID and WLR but not to WT. Accordingly, a significant reduction in lumen was observed in subjects under 52 years old concomitant to BP elevation (73.2 ± 9.0 for subjects with SBP >140 mmHg vs. 81.7 ± 10.2 μ m for subjects ≤ 120 mmHg) while this caliber reduction was not observed in older subjects (figure 1). Finally, WCSA was merely identical in the 3 BP groups (figure 1). In patients below 62 years, WCSA did not differ between BP tertiles indicating inward eutrophic remodeling.

Effects of antihypertensives treatment on retinal arteriolar remodeling

Short term effects of antihypertensive treatments were evaluated after a mean of 68 ± 46 days and 51 ± 22 days in the stable BP and in the BP drop group, respectively. Whereas no changes were observed in the group with a stable BP, in the group with a BP drop a significant decrease in SBP (-29.3 ± 17.3 mmHg) and DBP (-14.4 ± 10.0 mmHg) along with a decrease in WLR were observed, explained by an increase in retinal arteriolar ID without changes in WT (table 3). Moreover relative changes of BP, WLR and ID were significantly higher in the BP drop group (figure 2). In the later, antihypertensive drug regimen at follow-up compared to baseline consisted of significantly more drugs (2.1 vs. 1.5) of any classes (Additional table 3). Influence of long term antihypertensive treatments on retinal anatomical indices was evaluated using multivariate analysis on the whole population showing that being treated by any kind of treatment was not an independent correlates of nor WLR, ID, WT or WCSA (table 2). In a subgroup of subjects with office BP below 140/90 mmHg, no differences were observed in any retinal anatomical parameters between untreated normotensives subjects and hypertensives under a monotherapy whether treated by a RAS blocker or not (table 4).

Effects of other parameters on retinal arteriolar remodeling (Additional table 4).

Gender was an independent determinant of retinal anatomical indices: females exhibited higher WT and ID than males but with the same WLR. Diabetics exhibited hypertrophic remodeling with WLR increase mainly due to major WT and WCSA augmentation.

No changes were observed in dyslipidemic or obese patients.

Discussion

Adaptive optics camera is able to non-invasively and precisely assess the anatomy of retinal arteries. Thanks to AOC high precision, we were able to dissect WLR elevation to investigate the different underlying pathophysiological mechanisms. We observed inward eutrophic remodeling in hypertensives where BP acted to increase WT and to decrease ID. In ageing, we showed that WLR elevation was driven by WT increase independently of BP rise. We also observed diminution of BP elevation consequences on microcirculation anatomical indices with ageing. Concerning antihypertensive treatment effects on remodeling, although we did not observe additional drug actions beyond BP diminution, we observed on one hand that short-term drop in BP was accompanied by arteriolar ID enlargement and no WT decrease whereas on the other hand chronic antihypertensive treatment led to remodeling normalization.

Eutrophic remodeling of small arteries in hypertension has been widely studied in subcutaneous arteries with a 200 microns lumen [15] using Media to Lumen ratio and in the human retina arteries <100 microns[16] using the non-invasive Scanning Laser Doppler Flowmetry [17]. Several observations have led to the hypothesis that vasoconstriction could be the main trigger of inward remodeling beyond BP elevation itself. In opposition to large arteries where hypertension is associated with increased lumen diameter, in small arteries, ID is largely determined by local myogenic tone [18] which plays a key role in determining total peripheral resistance [16]. In vitro studies have shown that prolonged induced vasoconstriction led to arteriolar inward eutrophic remodeling [19–21] with involvement of vascular smooth muscle cells position shift [22], prolonged vasoconstriction, actin polymerization, transglutaminase activity and reactive oxygen species-dependent activation of matrix metalloproteinases [23]. Moreover, comparison of different antihypertensive drugs effects on BP and remodeling both in humans [24] and in hypertensive rats [25–27] showed that while all drugs successfully decreased BP, only drugs that induced vasodilatation, thus reducing vascular tone, could have a normalizing effect in terms of arteriolar remodeling. In line with these latter studies and others [28], we found that chronically treated and controlled hypertensives displayed the same retinal anatomical microvascular anatomy as untreated normotensives. Moreover, we were able to observe a decrease in WLR in case of a significant BP drop, attributed to a significant increase in ID with no changes in WT and WCSA, shortly after antihypertensive treatment introduction or reinforcement. This raises the question of the mechanisms of such modifications. We hypothesized that the short term changes observed here may be related to changes in vascular tone as a consequence of myogenic tone decrease due to antihypertensive drugs effect. This could explain our observations of a short term increase in ID parallel to BP drop. This hypothesis is comforted by the observation of remodeling triggered by vasoconstrictors or fully prevented by vasodilators[29] and suggests the important role of functional remodeling. Finally, the in-vivo strategy enables images to be acquired in conditions where microvascular arteries would display a degree of tone much different from those studied in a myograph.

However we made the observations that antihypertensive treatment had no independent influence on remodeling thus comforting the other hypothesis that BP itself could be the major trigger of remodeling. Actually, when BP varies, myogenic tone normally commands lumen to adapt to maintain sufficient flow. Therefore, a direct BP-driven WLR modifications could also be observed.

In our population, another main determinant of WLR was age, with age related increase in WT. Therefore, we extend to small arteries the previous demonstration that in large arteries, such as the aorta [30] or the carotid [31], ageing and hypertension had trophic effects on arterial wall. Concerning arterioles, previous imaging methods had already detected enlargement with age [32]. More recently, in line with our findings, studies using AOC showed the increase of WLR with age in small populations[33][34]. In addition, WLR anatomical components have been studied individually [6] indicating that aging is associated with a significant increase in WT while ID remained invariant.

In this large cohort, our results suggest a prominent role of structural remodeling during the ageing process. We also showed that if both age and BP acted to increase WT, BP elevation had fewer consequences on ID in older people. This may be explained by an age-related basal endothelial dysfunction mainly induced by the over-production and release of O₂⁻ and NO inactivation[35].

In diabetic patients we observed hypertrophic remodeling with significant increase in WT and WCSA, in line with the medial cross-sectional area augmentation described in subcutaneous small resistance arteries [36]. Interestingly, we observed harmonious elevation of ID and WT in females without changes in WLR. The first explanation might be a smaller eye depth in

women leading to ID and WT calculation errors. However, although we did not record hormonal status, when focusing on females over 50 years, such significant differences did not persist (data not shown). Estrogen complex effect on microcirculation could explain ID elevation. Estrogen regulates arteriolar responses to pressure in both physiological and pathological conditions to elicit a lower basal tone of microvessels via a nuclear and a membrane form of estrogen receptors. In the nucleus, estrogen promotes genes expression up-regulation leading to synthesis of endothelial nitric oxide synthase, cyclooxygenase, antioxidant enzyme superoxide dismutase, and a down-regulation of NADPH oxidase in the cytoplasm. In the cytoplasm, estrogen triggers the synthesis of series of target proteins and acts as a Ca^{++} -channel blocker. Therefore, the presence of estrogen favors an attenuation of pressure-induced constriction and an augmentation of flow-induced dilation, as a consequence of increased release of endothelial vasodilators [37]. The harmonious WT and ID elevation may therefore be attributed to normalization of parietal stress.

Our study exhibits some limitations. First, WLR has no dimension whereas WT and ID are distances measured in microns. No formal calibration on phantoms has been made and distances were calculated using angle measure coupled with a standard eye depth of 24mm. Due to heterogeneity in eye depth it may have led to over or underestimation of distances in individuals. The large sample of our population tends to minimize those individual variations. Moreover, we previously validated our measures against already published data with Scanning Laser Doppler flowmetry. Furthermore, we did not use exact coordinates to verify the localization of repeated measures. However, retinal arteriolar conformation is unique and proper to each individual so the double checking by blinded observer and the fact that measures were obtained from a segment length and not on a particular point limited possible errors.

Our study showed that although high BP and age are associated with retinal arterioles' WLR elevation. The different underlying mechanisms can now be discriminated by AOC indicating that retinal arteriolar remodeling comprised BP and age-driven wall thickening as well as BP-triggered lumen narrowing in younger subjects. Moreover, we differentiated the chronic remodeling effects of BP and ageing from the short-term changes in arteriolar ID observed parallel to BP changes. Finally, we hypothesized that remodeling reversal observed in controlled hypertensives seems to include short-term functional and long-term structural changes.

In humans, the rtx1® using adaptive optical camera is a helpful non-invasive method that complements the traditional histological ex-vivo studies by enabling large screening and consecutive measurements. It opens the way to assess the selective effects of antihypertensive or antidiabetic drugs on microcirculation at different time points as well as prospective studies on cardiovascular and cerebrovascular risk in hypertensive subjects.

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Alessandro Mattina: OA image acquisition and analysis, study realization and statistics

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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TABLES

Global Population	
Subjects number	1000
Age.- years	56.1 ± 12.8
Gender .- Male	514 (51.4%)
BMI.- kg/m ²	27.3 ± 5.7
Office BP	
SBP.-mmHg	130.4 ± 20.4
DBP.-mmHg	71.8 ± 13.6
Retinal Arteriolar Microvasculature	
Wall to Lumen Ratio	0.300 ± 0.054
Internal Diameter.- μm	78.0 ± 10.9
Wall Thickness.- μm	23.2 ± 3.9
Wall Cross Sectional Area. -μm ²	3293 ± 856
Cardiovascular Risk Factors	
Hypertension. -n (%)	613 (61.3%)
Dyslipidemia. -n (%)	456 (45.6%)
Obesity. -n (%)	265 (26.5%)
Diabetes. -n (%)	186 (18.6%)
Current smoking. -n (%)	148 (14.8%)

SBP,DBP are systolic and diastolic pressures

Table 1. Subjects description including basic characteristics, blood pressure measurements, anatomical microvascular indices and risk factors

	Model R ²	SBP (β ± SD)	Age (β ± SD)	Covariates
WLR	0.18 ‡	0.0009 ± 0.00009 ‡	0.0007 ± 0.0001 ‡	gender, Diabetes, (SBPxAge) ‡, BMI, Treatment
Wall.- μm	0.15 ‡	0.044 ± 0.007 ‡	0.072 ± 0.011 ‡	gender ‡, Diabetes ‡, (SBPxAge), BMI, Treatment
Lumen.- μm	0.06 ‡	- 0.078 ± 0.020 ‡	0.059 ± 0.031	gender ‡, Diabetes ‡, (SBPxAge) ‡, BMI, Treatment
WCSA. -μm ²	0.09 ‡	4.0 ± 1.5 ‡	13.9 ± 2.44 ‡	gender ‡, Diabetes ‡, (SBPxAge), BMI, Treatment

WLR is Wall to lumen Ratio, WCSA is wall cross sectional area, SBP is Systolic Blood Pressure , * p <.05 , ‡ p<.01 ‡ p<.001

Table 2. Correlates of Wall Thickness, Internal Diameter, Wall-to-Lumen Ratio and Wall Cross Sectional Area.

	Stable BP Group (n=30)		BP Drop Group (n=45)	
	Baseline	Follow up	Baseline	Follow up
Office Blood Pressure				
Systolic BP.- mmHg	139.9 ± 19.8	137.3 ± 18.9	157.5 ± 24.3	128.2 ± 20.1 ‡
Diastolic BP.- mmHg	79.5 ± 15.3	78.0 ± 13.0	86.4 ± 14.8	72.0 ± 10.1 ‡
Retinal Microvasculature				
WLR	0.304 ± 0.054	0.307 ± 0.057	0.342 ± 0.072	0.321 ± 0.070 ‡
Lumen diameter.- µm	75.4 ± 11.9	75.7 ± 12.3	73.3 ± 8.8	76.2 ± 9.8 ‡
Wall Thickness.- µm	22.7 ± 4.0	22.9 ± 4.1	24.7 ± 4.7	24.3 ± 5.4
Wall Cross Sectional Area.- µm ²	3198 ± 988	3190 ± 981	3352 ± 943	3412 ± 1075

Data are expressed as Mean ± SD, BP is Blood Pressure, ‡ p<.0001 between baseline and follow-up (t test on matched pairs)

Table 3. Baseline and follow-up Blood Pressures and microvascular anatomical indices in the Stable and Drop BP groups

	Controlled BP			ANOVA
	Untreated	Monotherapy		
	All	No RAS blocker	RAS blocker	
N.	387	43	66	
Age	52.5 ± 13.3	54.1 ± 14.6	59.3 ± 9.2 *†	<.0001
Office Blood Pressure				
Systolic BP.- mmHg	117.7 ± 12.9	123.7± 10.0 *	122.9 ± 11.5 *	<.0001
Diastolic BP.- mmHg	67.1 ± 10.3	68.9 ± 10.9	68.6 ± 9.8	0.33
Retinal Microvasculature				
WLR	0.285 ± .055	0.299 ± 0.046	0.294 ± 0.050	0.11
Lumen diameter.- μm	79.0 ± 11.4	77.4 ± 15.4	79.1 ± 12.0	0.67
Wall Thickness.- μm	22.2 ± 3.6	23.1 ± 4.1	22.9 ± 3.4	0.17
Wall Cross Sectionnal Area.- μm²	3180 ± 790	3329 ± 1057	3292 ± 823	0.35

Data are expressed as Mean ± SD, BP is Blood Pressure, * p <.05 compared to untreated normotensives, † p<.05 compared to Ras blocker group

Table 4 Age, Blood Pressure and retinal anatomical indices in untreated normotensives and in hypertensives subjects treated by a monotherapy and with controlled Blood Pressure.

FIGURES

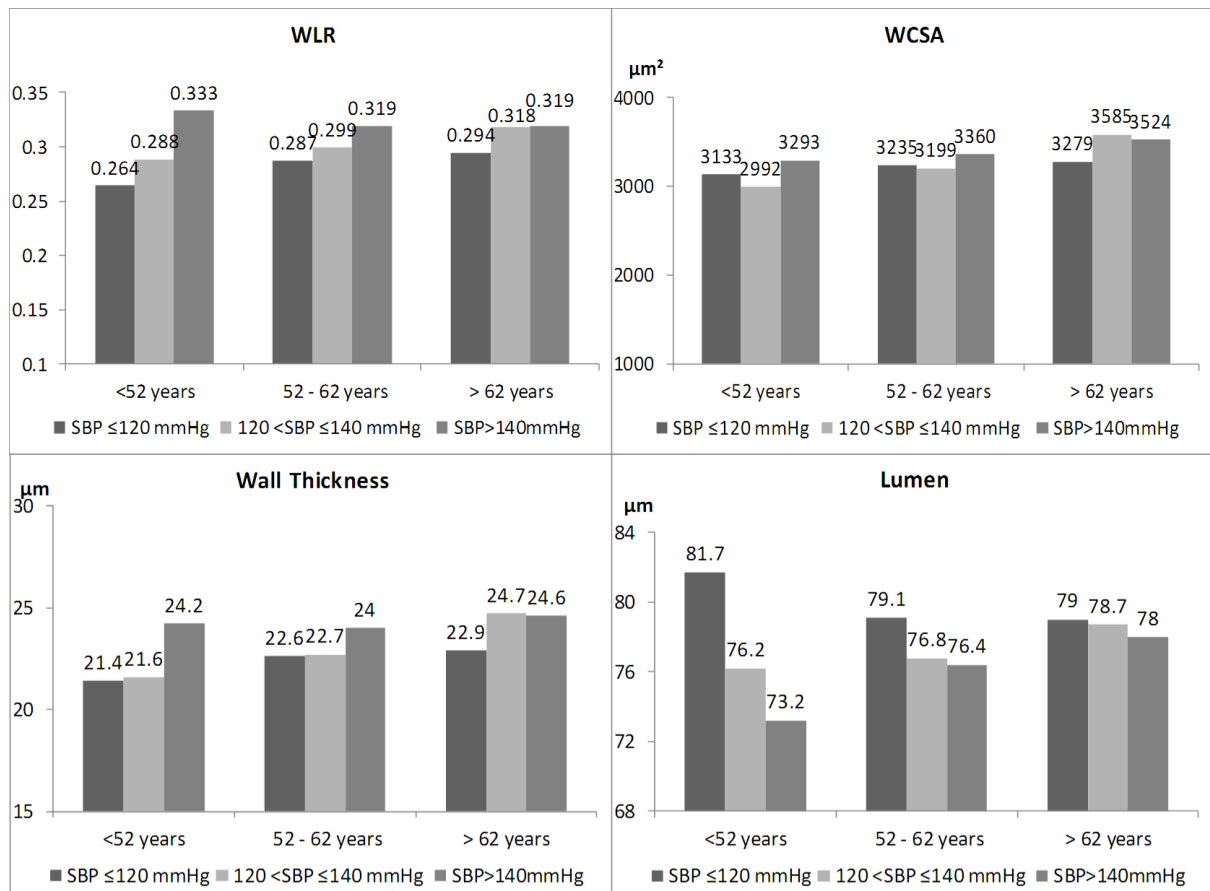


Figure 1.

Wall Thickness, Lumen, Wall-to-Lumen Ratio (WLR) and Wall Cross Sectional Area (WCSA) means according to age tertiles and Blood Pressure level. 2 ways ANOVA was significant for all parameters ($p < .0001$). Blood Pressure effect and interaction between age and Blood Pressure effect were significant for all parameters. Age effect was significant for WLR, WT and WCSA.

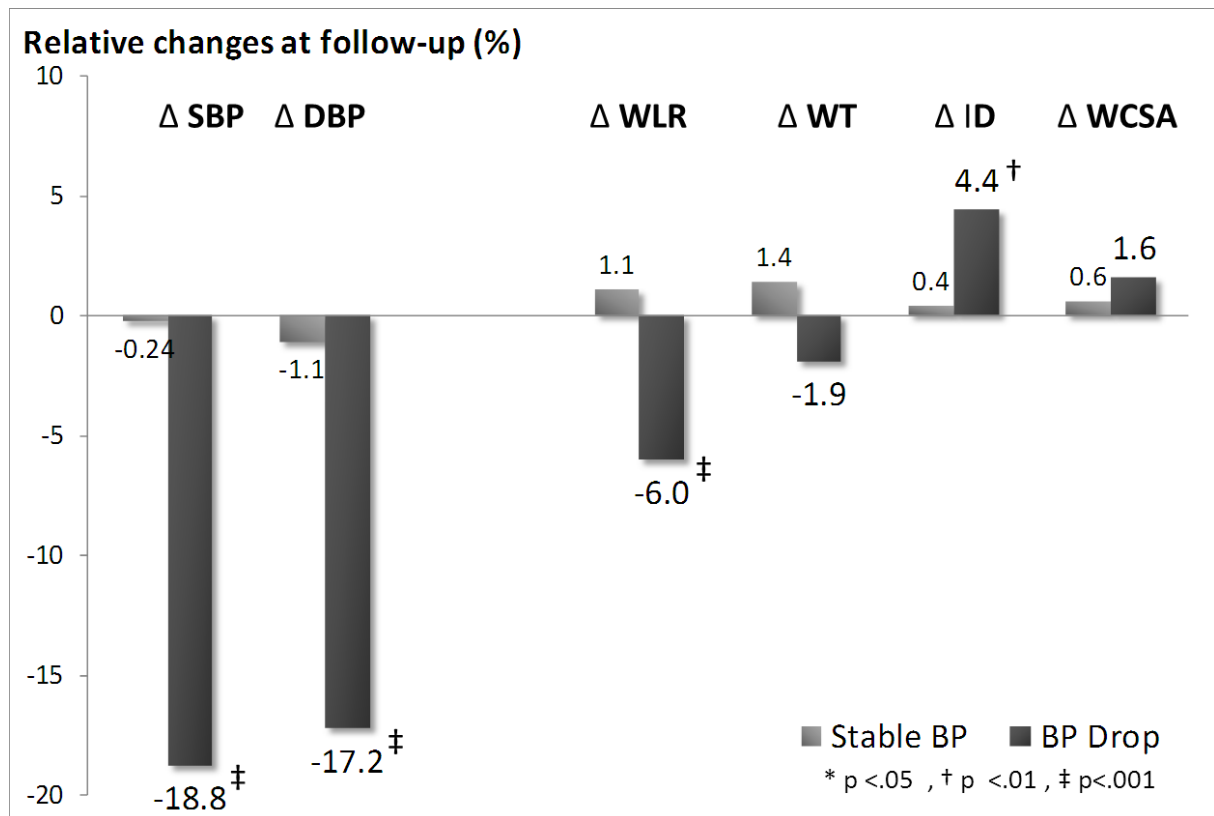


Figure 2.

Relative changes (%) between baseline and follow-up assessment of retinal microvasculature anatomical indices as well as systolic and diastolic BP. Statistical significance between groups is indicated by * p value <.05, † p value <.01, ‡ p<.001. SBP is systolic blood pressure, DBP is diastolic blood pressure, WLR is Wall to Lumen ratio, ID is internal diameter, WT is Wall thickness, WCSA is Wall Cross Sectional Area.

4. Discussion générale

Dans cette thèse, nous avons plusieurs objectifs :

- 1) Etudier les relations entre âge et hypertension dans les grosses artères
- 2) Déterminer des liens entre fonction et anatomie au niveau des grosses artères
- 3) Valider un nouvel outil pour observer l'anatomie des altérations microvasculaires rétinienne dans l'hypertension
- 4) Etudier les relations entre le remodelage microvasculaire rétinien et des indices hémodynamiques ainsi que d'anatomie et de fonction des grosses artères dans l'hypertension
- 5) D'étudier les effets respectifs de l'âge, de la pression artérielle et des traitements antihypertenseurs sur les déterminants du remodelage microvasculaire rétinien.

4.1 Influence de l'âge et de la pression sur les grosses artères

Dans notre travail, en utilisant l'IRM, nous avons pu observer une augmentation du diamètre, de la hauteur, de la longueur et de la largeur de l'aorte ascendante chez les patients hypertendus en comparaison avec les témoins. De plus, la largeur de l'arche aortique était plus importante chez les hypertendus non contrôlés par rapport aux hypertendus contrôlés. Après ajustement sur l'âge, le niveau tensionnel n'était plus un déterminant indépendant de la géométrie aortique. En ce qui concernant la distensibilité de l'aorte ascendante, nous avons redémontré dans cette population d'hypertendus ce qui avait déjà été montré dans plusieurs études de cohorte : c'est-à-dire qu'à la fois le vieillissement et l'augmentation de la pression artérielle contribuent chacun de façon indépendante à augmenter la rigidité.

4.2 Relations structure/fonction dans les grosses artères

Nous avons démontré au sein d'une large population de patients avec des facteurs de risque qu'il existait un lien entre un marqueur des ondes de réflexion au niveau de l'artère radiale et le fardeau athérosclérotique carotidien. Par ailleurs nous avons pu démontrer que ce lien était indépendant de la présence de facteurs de risque cardiovasculaires et notamment de l'âge. Deux petites études portant sur de petites populations sélectionnées de patients avaient

déjà montré une association entre une augmentation de l'AI et des marqueurs d'athérosclérose [90,91]. Notre travail est le premier à montrer un lien entre l'AI radial et athérome dans une large population en prévention primaire. Nos résultats suggèrent que les ondes de réflexion pourraient être en partie responsables de l'augmentation du risque cardiovasculaire en favorisant le développement de l'athérosclérose et qu'elles pourraient expliquer les liens démontrés entre Ai et événements cardiovasculaires [92]. L'hypothèse avancée jusqu'à présent était que la rigidité augmentait la pression pulsée ce qui générerait une surcharge de pression sur le ventricule gauche et une diminution de la perfusion coronaire. Un contre argument est celui que la majorité des événements constituant le « risque cardiovasculaire » sont des événements d'origine athéromateuse (en particulier les syndromes coronariens aigus) et non de l'insuffisance cardiaque. Ainsi, nos résultats permettent de proposer que les réflexions d'ondes puissent causer une augmentation de la contrainte de cisaillement oscillatoire dans la paroi artérielle. En effet, cette dernière a été montrée comme pouvant favoriser la progression de l'athérosclérose. En outre, Beaussier et al.[93] ont pu démontrer qu'à l'endroit des plaques survenait une contrainte de flexion vers l'intérieur qui pouvait à son tour renforcer la croissance de la plaque et/ou sa rupture en induisant un stress cellulaire[94] et/ou de la fatigue mécanique.

4.3 Relations entre territoires micro et macrovasculaires

En étudiant de façon non invasive dans une petite population de patients hypertendus les relations entre le remodelage microvasculaire rétinien et l'anatomie et la fonction des grosses artères nous avons observé que les altérations observées en parallèle dans ces 2 territoires vasculaires étaient médiées par les effets généraux combinés de l'âge et de la pression. Nous avons aussi pu montrer que le diamètre de l'aorte ascendante était corrélé au remodelage microvasculaire et ce de façon indépendante de la pression et de l'âge.

Peu de données existent sur les relations entre petites et grosses artères chez les hypertendus. Nous retrouvons dans notre population ce qui avait déjà été publié dans une population générale, où le rétrécissement des artérioles rétiniennes évalué par le rapport artériole/veines mesuré sur une photographie du fond d'œil avait pu être relié au diamètre ainsi qu'à la distensibilité de l'aorte indépendamment de la pression artérielle systolique [95]. Par ailleurs, Salvetti et al [32] ont constaté que la VOP-cf pouvait être un déterminant indépendant du remodelage rétinien (mesuré par le WLR) dans une population hétérogène de

patients diabétiques et d'hypertendus (traités et jamais traités). Dans notre travail, alors que les indices de rigidité étaient associés à un remodelage eutrophique rétinien en analyse univariée, ces associations n'étaient plus significatives après ajustement pour l'âge et la pression. En revanche, les résistances périphériques étaient le seul déterminant indépendant du WLR, expliquant 44% de la variance du modèle multivarié expliquant le WLR par l'âge, l'IMC, les traitements antihypertenseurs, la pression systolique centrale et les résistances périphériques.

Bien que plusieurs méthodes non invasives aient été proposées pour évaluer le débit cardiaque, la méthode de thermo-dilution directe reste la référence pour évaluer le débit cardiaque mais nécessite un cathétérisme. Récemment, un dispositif d'impédancemétrie non invasif a été validé chez des patients en soins intensifs mais la précision de ce type de méthode est contestée. Ici, nous avons utilisé une méthode entièrement non-invasive pour calculer la résistance en combinant des mesures de pression centrale avec le SphygmoCor Xcel et une mesure directe du débit aortique par l'IRM. Cette nouvelle méthode de calcul non invasif des TPR permet son application en pratique clinique et/ou à plus large échelle.

4.4 Influence de l'âge, des facteurs de risque cardiovasculaires et des traitements antihypertenseurs sur le remodelage microvasculaire

L'optique adaptative est en mesure d'évaluer précisément de façon non invasive et à grande échelle l'anatomie des artères rétiniennes. Nos principaux résultats obtenus avec cette technique sont cohérents avec les résultats antérieurs montrant différents types de remodelage microvasculaire dans l'hypertension (remodelage eutrophique centripète) et dans le diabète (remodelage hypertrophique). En outre, nous avons observé un remodelage secondaire au vieillissement dû à une augmentation isolée de l'épaisseur pariétale. Nous avons pu montrer par ailleurs un remodelage « harmonieux » chez les sujets de sexe féminin qui présentaient un WLR identique à celui des hommes mais avec une épaisseur et un diamètre plus importants.

Concernant les traitements antihypertenseurs nous avons pu montrer qu'une baisse à court terme de la pression artérielle induit par une intensification thérapeutique s'accompagnait d'une diminution du WLR secondaire à un élargissement de la lumière artériolaire sans changement de l'épaisseur pariétale ni de la surface sectionnelle du vaisseau. Par ailleurs, une « normalisation » du WLR et des autres indices anatomiques artériolaires

réiniens a pu être observée chez les hypertendus traités et contrôlés sur du long terme et ce de façon indépendante du type de traitement antihypertenseur.

Le remodelage eutrophique centripète des petites artères dans l'hypertension a été largement étudié en utilisant le rapport Media/ Lumen (MLR) dans les artères sous-cutanées ayant une lumière d'environ 200 microns. Ceci a également été observé sur les artères de rétine humaine en utilisant la technique de SLDF et ce sur ces artères de moins de 100 microns de diamètre, là où les résistances se produisent. Notre travail sur plus de 1000 sujets permet de confirmer ces résultats.

Plusieurs observations ont conduit à l'hypothèse que la vasoconstriction pourrait être le déclencheur principal du remodelage, au-delà de l'élévation de la pression elle-même. Tout d'abord, *in vitro*, la vasoconstriction induite prolongée peut conduire à un remodelage eutrophique artériolaire [96] via avec des processus qui impliquent des changements de position des cellules musculaires lisses [97], la polymérisation de l'actine, l'activité de la transglutaminase et du stress oxydant ou l'activation des métalloprotéinases de matrice [98]. En outre, la comparaison des différents effets de médicaments antihypertenseurs sur la pression artérielle et le remodelage à la fois chez l'homme [99] et chez les rats hypertendus [100–102] a montré que, bien que tous les médicaments diminuent la pression, seuls les médicaments qui ont induit une vasodilatation, réduisant ainsi le tonus vasculaire, pourraient avoir un effet de « normalisation » du remodelage artériolaire.

Dans notre population, l'épaisseur pariétale augmentait avec l'âge chez les normotendus ainsi que chez les sujets hypertendus. Nous avons ainsi montré qu'à la fois l'âge et la pression artérielle avaient pour effet d'augmenter l'épaisseur pariétale. Des méthodes d'imagerie antérieures avaient déjà détecté l'élargissement du diamètre total des artéioles avec l'âge [103], mais ces techniques ne possédaient pas une résolution suffisante pour attribuer cette augmentation à une augmentation de la lumière ou de l'épaisseur pariétale. Notre travail permet de montrer précisément grâce à l'optique adaptative que cet élargissement est principalement dû à une augmentation de l'épaisseur pariétale sans changement du diamètre luminal. L'effet du vieillissement et de l'hypertension sur l'épaisseur pariétale a été largement démontré chez l'animal et chez l'homme en particulier dans les grosses artères, tels que l'aorte [104] ou l'artère carotide [105].

Dans notre population, le diamètre interne du vaisseau n'était pas modifié avec l'âge conduisant à l'hypothèse que l'augmentation observée de l'index de remodelage avec l'âge est en fait secondaire à un épaississement pariétal en raison de modifications structurelles du mur vasculaire. A l'opposé, en analysant les déterminants de l'augmentation du WLR dans l'hypertension nous avons observé à la fois une augmentation de l'épaisseur pariétale et une diminution de la lumière interne sans changement de la surface sectionnelle du vaisseau. De plus, l'âge n'influait pas le diamètre alors qu'une augmentation de pression était indépendamment corrélée à une diminution du calibre interne de la lumière. Cette influence de la pression était surtout observée chez les sujets les plus jeunes de notre population (<52 ans). Ceci peut être éventuellement expliqué par la dysfonction endothéliale liée à l'âge secondaire à l'hyperproduction de O₂- et à l'inactivation du NO [106]. Dans les grosses artères nous avons vu que et l'âge et l'hypertension étaient associées à une augmentation diamètre de la lumière. Notre travail suggère qu'à l'inverse, dans les petites artères, la lumière serait déterminée par le tonus myogénique local [107], qui joue un rôle clé dans la détermination des résistances périphériques totales [108] et donc du niveau tensionnel. Lorsque la pression chute, le tonus myogène commande normalement que la lumière puisse augmenter pour maintenir un débit suffisant. Quelques études antérieures sur le remodelage microvasculaire ont montré des modifications du MLR [109] et du WLR [110] sous traitement, bien que dans cette dernière étude, le diamètre interne n'était pas significativement accru après un traitement de 4 semaines par lercanidipine. Ici, grâce à la reproductibilité et à la haute précision de l'optique adaptative, nous avons pu montrer une diminution du WLR en cas de baisse significative de la pression au cours d'un suivi à court terme. Cette diminution a été attribuée à une augmentation significative du diamètre interne sans changement d'épaisseur pariétale ni de surface sectionnelle du vaisseau. La part relative fonctionnelle et structurelle de ces modifications ne peut pas être expliquée de nos données. D'un point de vue physiopathologique, l'augmentation de la lumière peut être expliquée en partie par l'inhibition du système rénine angiotensine dont nous avons détaillé plus haut les implications importantes dans les processus mis en jeu dans le remodelage de la paroi vasculaire. Prises ensemble, les modifications que nous visualisons à court terme ainsi que l'observation de modifications rapides des rétrécissements artériolaire rétinien [111], l'absence de corrélation entre le diamètre avec l'âge et l'observation d'un remodelage totalement empêché par des composés vasodilatateurs [112] suggèrent un rôle prééminent du remodelage « fonctionnel » dans l'hypertension.

Pris tout ensemble ces résultats pourraient conduire à considérer le WLR comme marqueur global des 2 remodelages microvasculaires: l'un structurel et l'autre fonctionnel. D'un côté, l'épaisseur pariétale pourrait représenter la structure, sous l'influence du vieillissement, et de la pression artérielle. D'un autre côté, le diamètre interne du vaisseau pourrait représenter la partie fonctionnelle du remodelage, influencée par la pression et le tonus myogénique local (Figure 8 Déterminants du Wall-to-Lumen-Ratio

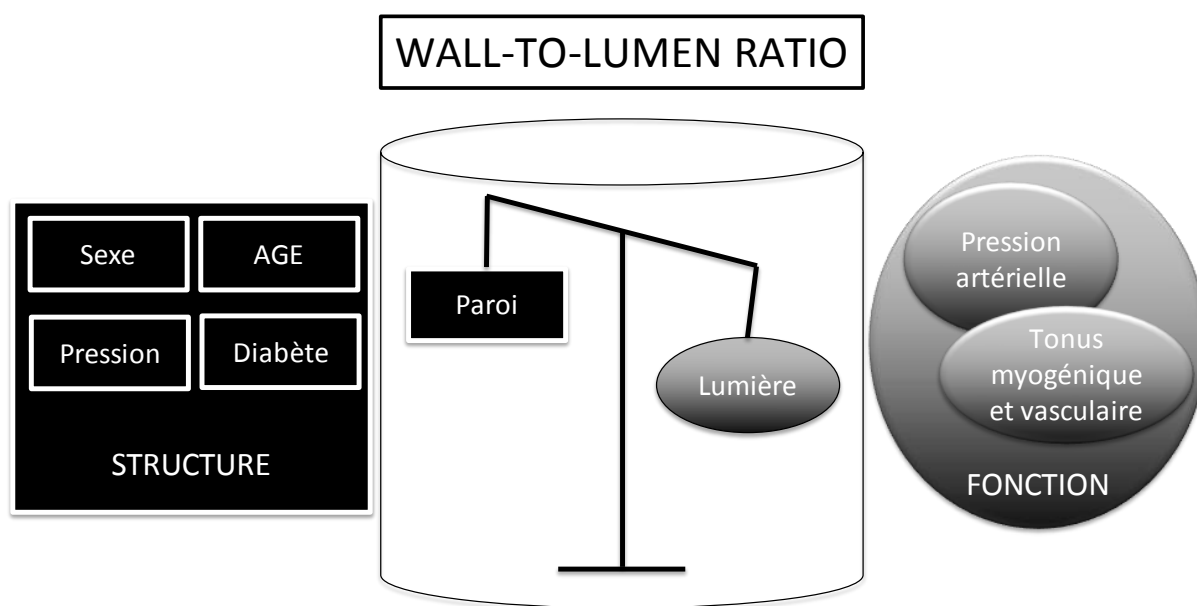


Figure 8 Déterminants du Wall-to-Lumen-Ratio

En regardant la table 6 ci-dessous, on peut constater que le paramètre qui pourrait peut-être le mieux discriminer les différents types de remodelage est la surface sectionnelle du vaisseau.

	WLR	Epaisseur pariétale	Diamètre artériolaire	Surface trans sectionnelle
Hypertension	+	+	-	=
Vieillessement	+	+	=	+
Diabète	+	++	+	++
Sexe féminin	=	+	+	+

Table 6 Facteurs associés à des variations des indices anatomiques microvasculaires rétiens

Nous avons montré qu'elle n'était pas augmentée chez les patients présentant une hypertension artérielle essentielle et qu'elle n'était pas modifiée à court terme en cas de baisse

tensionnelle importante induite par un traitement antihypertenseur. Depuis la soumission des articles, nous avons pu recueillir les données vasculaires rétinienne de 59 sujets hypertendus traités après 40 semaines de suivi. Parmi eux, 28 présentaient une pression artérielle stable et 29 avaient présenté une baisse significative de pression artérielle sous traitement antihypertenseur (Figure 9 Variations relatives à long terme en % des pressions systoliques et diastoliques (SBP et DBP) ainsi que des indices anatomiques (WLR : rapport mur/lumière, WT : épaisseur pariétale, ID : lumière et WCSA : surface transsectionnelle) dans les 2 groupes de patients * $p<0.0001$; † $p<0.001$; £ $p<0.01$; ‡ $p<0.05$). A 40 semaines, ces derniers présentaient par rapport au départ, une diminution de 11% du WLR due à une augmentation de 5.6% de la lumière et une diminution de 6.5% de l'épaisseur de la paroi. En revanche, la surface de la paroi vasculaire, elle, inchangée. Sous traitement antihypertenseur, il s'est donc produit un remodelage eutrophique à l'exact inverse de celui décrit dans l'hypertension. Le fait que la surface observée du vaisseau et donc par extension, son « volume de paroi » demeure inchangé renforce fortement l'hypothèse qui est celle que les mécanismes à l'œuvre soient plus liés au tonus et à des réarrangements structuraux de la paroi plutôt que des modifications de la « masse » vasculaire.

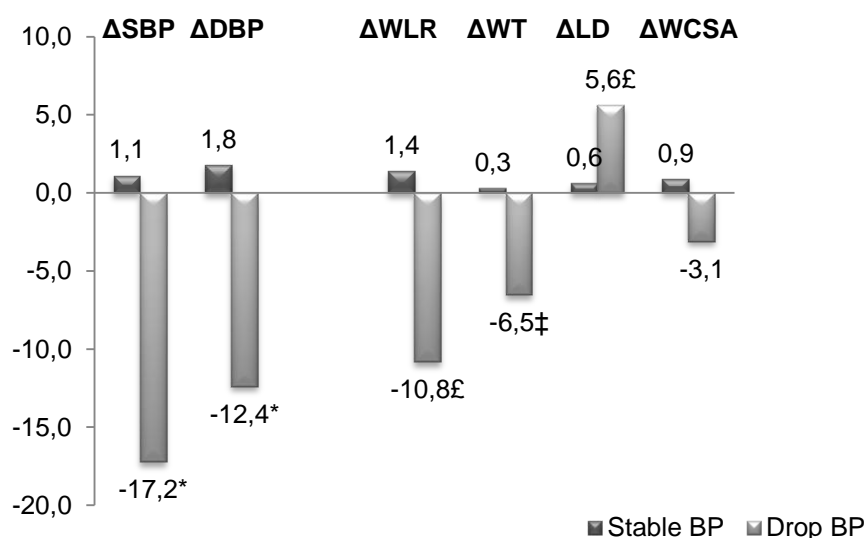


Figure 9 Variations relatives à long terme en % des pressions systoliques et diastoliques (SBP et DBP) ainsi que des indices anatomiques (WLR : rapport mur/lumière, WT : épaisseur pariétale, ID : lumière et WCSA : surface transsectionnelle) dans les 2 groupes de patients
* $p<0.0001$; † $p<0.001$; £ $p<0.01$; ‡ $p<0.05$

5 Conclusion

5.1 Résumé

Dans ce travail nous avons cherché à discerner les rôles respectifs de l'âge et de l'hypertension sur l'anatomie et la fonction des grosses et des petites artères.

En ce qui concerne les grosses artères nous avons pu confirmer les résultats précédemment obtenus par d'autres techniques ou dans d'autres populations en utilisant une imagerie permettant une approche de physiologie non invasive dans une population sélectionnée de patients hypertendus. Ainsi, concernant l'anatomie des grosses artères, nous avons montré le rôle proéminent de l'âge et nous avons observé l'influence parallèle de l'âge et de la pression artérielle sur la diminution de la rigidité artérielle.

Par ailleurs en utilisant la tonométrie au niveau de l'artère radiale nous avons pu mettre en évidence des liens anatomie/fonction au niveau des grosses artères dans une large population en prévention primaire.

En ce qui concerne la microcirculation, nous avons aussi utilisé et validé une nouvelle technologie d'imagerie non invasive de haute précision qui nous a permis d'étudier en détail les déterminants du remodelage artériolaire rétinien dans l'hypertension. Nous avons ainsi observé dans une large cohorte les rôles respectifs de l'âge et du niveau tensionnel. Ainsi, si les 2 favorisent de façon parallèle l'épaississement des parois artériolaires, seul le niveau tensionnel était responsable de façon indépendante de la diminution de calibre de la lumière artérielle.

Grace à la précision et à la reproductibilité de l'optique adaptative nous avons observé des variations à court terme du diamètre interne artériolaire en cas de diminution de pression. Conjugués à la corrélation que nous avons pu établir entre WLR et résistances périphériques totales, ces résultats plaident en faveur d'une part importante de remodelage fonctionnel dans l'hypertension. A l'inverse, le vieillissement serait lui responsable d'un remodelage structural touchant la paroi du vaisseau. Enfin, la prise en compte de la surface du vaisseau semble capitale pour mieux discriminer les processus de remodelage à l'œuvre.

5.2 Conclusion et perspectives.

Au terme de nos travaux on peut conclure que si l'âge et la pression artérielle exercent des effets similaires à première vue sur le système artériel, une étude plus approfondie permet de dissocier leurs effets respectifs, en particulier sur les remodelages structurels et fonctionnels microvasculaires. Tout ceci a été rendu possible non seulement par tous les travaux précurseurs de nos prédécesseurs mais aussi par les progrès techniques réalisés ces dernières années et notre travail s'inscrit dans cette double lignée.

Nous avons pu valider et démontrer l'utilité des nouvelles méthodes d'imagerie et d'analyse artérielle ce qui nous a permis de pénétrer plus profondément dans la compréhension des mécanismes en jeu dans le vieillissement et dans l'hypertension. Cette approche de physiologie non invasive est complémentaire d'une approche « in vitro » en ce qu'elle permet des études à grande échelle et de façons itératives chez un même sujet.

Les possibilités de ces nouvelles techniques, combinées aux hypothèses générées par notre travail, ouvrent de nouvelles voies d'explorations pour l'avenir. La première serait de savoir s'il est possible d'observer et de mesurer une pulsation du mur artériolaire (la théorie nous dit que non, mais aucune technique n'a jamais été en mesure de le montrer). Toujours dans une idée exploratoire, notre travail s'est limité à une petite partie de l'arbre artériolaire rétinien, peut être aurions-nous intérêt à répéter nos analyses plus en distalité et en particulier au niveau des capillaires mais ceci reste pour le moment difficile pour des raisons techniques et anatomiques. On pourrait aussi s'intéresser à la régularité des parois artérielles, à l'anatomie des bifurcations, aux variations de localisation des rétrécissements focaux, et surtout effectuer des expériences permettant de faire varier le tonus myogénique. Dans une optique plus médicale, il serait intéressant de pouvoir comparer les effets de différentes thérapies antihypertensives sur le remodelage microvasculaire rétinien, à court/moyen et long terme ainsi que d'utiliser l'optique adaptative pour étudier l'intérêt prédictif du remodelage rétinien dans le risque cardiovasculaire et en particulier cérébrovasculaire.

Nous concluons en rappelant que s'il a fallu 8 ans pour que les calculs de Le Verrier se voient confirmés par l'observation de Neptune par Johann Galle, à l'inverse les progrès technologiques rapides de notre époque nous permettent de voir ce que nous ne comprenons pas encore. Il est important que science et technologie puissent continuer à avancer ensemble

pour nous permettre d'aller toujours plus loin dans la compréhension des phénomènes du vivant.

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Résumé :

Dans ce travail nous avons cherché à discerner les rôles respectifs de l'âge et de l'hypertension sur l'anatomie et la fonction des grosses et des petites artères en utilisant de nouvelles techniques non invasive d'exploration artérielle.

1/Au niveau macrovasculaire :

En utilisant l'IRM pour étudier l'aorte nous avons pu confirmer dans une population de patients hypertendus les résultats précédemment obtenus par d'autres techniques ou dans d'autres populations. Ainsi, concernant l'anatomie des grosses artères, nous avons montré le rôle proéminent de l'âge et observé l'influence parallèle et combinée de l'âge et de la pression artérielle sur la diminution de la rigidité artérielle. Par ailleurs en utilisant la tonométrie au niveau de l'artère radiale nous avons pu mettre en évidence des liens entre anatomie, athérosclérose et fonction artérielle dans une large population en prévention primaire avec des facteurs de risque suggérant de nouvelles hypothèses pour relier fonction artérielle et risque cardiovasculaire

2/Au niveau microvasculaire :

En ce qui concerne la microcirculation, nous avons aussi utilisé et validé une nouvelle technologie d'imagerie non invasive de haute précision : l'optique adaptative. Cela nous a permis d'étudier en détail les déterminants du remodelage artériolaire rétinien dans différentes situations

(1) Nous avons confirmé dans une très large cohorte d'hypertendus la présence d'un remodelage eutrophique. Grâce à la précision et à la reproductibilité de l'optique adaptative nous avons observé des variations à court terme de la lumière artériolaire sans modification de la surface sectionnelle du vaisseau en cas de diminution de pression. Conjugués à la corrélation que nous avons pu établir entre l'indice de remodelage rétinien et les résistances périphériques totales, nos résultats plaident en faveur d'une part importante de remodelage fonctionnel dans l'hypertension.

(2) A l'inverse, avec le vieillissement, nous avons pu décrire un remodelage consistant en un épaissement pariétal avec augmentation de la surface sectionnelle sans modification de la lumière artérielle.

(3) Nous avons confirmé dans une large cohorte la présence d'un remodelage hypertrophique chez les patients atteints de diabète de type 2.

En conclusion :

Nous avons validé et démontré l'apport de nouvelles techniques d'imagerie artérielle non invasives pour étudier sur de larges populations les effets le remodelage artériel dû à l'âge et aux facteurs de risque cardiovasculaires. Cette approche de physiologie in vivo pourrait s'avérer très utile en pratique clinique quotidienne pour l'évaluation et le suivi thérapeutique des patients présentant une hypertension ou des facteurs de risque cardiovasculaire.

Mots clefs : hypertension, vieillissement, remodelage, microcirculation, optique adaptative, IRM